

Metrication and the falling yen

The General Accounting Office (GAO) in Washington is plainly as puzzled as the rest of the world that so little has happened to make the metric system a reality in the United States. The falling yen will prolong the puzzle.

HISTORICALLY, the surprise is that the United States did not go metric at the outset, given the French influence on the conduct of the War of Independence that threw out the British. Since then, there have been many false starts. Congressional procedures have, over the years, encouraged a steady stream of bills aimed at rationalizing the irrational, making it easier for those who learned to count in tens also to know by how much they are breaking the speed-limit (still a maximum of 55 miles an hour) or, more usefully, to translate a tonnage of oil spilled off the coast of Alaska into a volume. The latest development, and that which has stirred GAO into action, is an amendment tagged onto the Trade and Competitiveness Act of 1988 (which also sanctions US action against unfair trading partners) giving federal agencies until 1992 to use the metric system in their procurement of supplies.

What emerges from GAO's enquiry is simple; the administration does not have its heart in metrication. Not that the bureaucracy has been entirely neglectful. The Department of Commerce has been nominated as the "lead agency", and has dutifully set up two interagency committees. The department has also provided terms of reference for ten other committees, and has appointed chairmen for nine of them. But only one committee (that for the construction industry) has met — on three occasions. If there are white sheep among the 37 agencies surveyed, they are the Nuclear Regulatory Agency (which hopes to be finished in 1997) and the Department of Defense (which has more than a single official working on the project).

GAO seems also to have identified a sense of urgency at the National Aeronautics and Space Administration (NASA), which is reported to be concerned that the multinational space station should fit properly together even though some parts, the *Hermes* spacecraft, for example, will be built in France. With characteristic prudence, NASA estimates that the cost of metrication on the space station will be \$200 million. A few more numbers like that, and even the Congress will be found to be cooling on the whole idea.

What GAO now wonders is whether the Department of Commerce should be given stronger powers to coerce federal agencies to meet deadlines it imposes. That, no doubt, would help, but not much. The plain truth is that the United States is not yet ready for metrication. Those who trade corn (in bushels) or buy gasoline (in gallons)

are too fond of their present ways. It might well have been different if the yen were not falling with the Tokyo Stock Exchange, or if Mrs Carla Hills, the US Trade Representative, had not last week won her well-advertised and well-staged battle with the Japanese over trade. The truth is that US alarm about competitiveness is not constant. What seems to matter — and to hurt — are the mutually cancelling trade deficits and the scale of Japanese investment in US real estate. Now, no doubt temporarily, both are falling, so that competitiveness and metrication with it are pushed into the background. But the falling yen means that the trade deficit will soon increase again, when metrication will no doubt come into its own once more. Meanwhile, the biggest danger is that the construction industry's zealous committee will make such progress, and the others will make so little, that building accessories will not fit together as they should. □

Jones bows out

The Australian government has lost an able minister with the decision to drop its science minister.

THE departure of Mr Barry Jones, minister of science in the Australian government until last week, when he was dropped against his wishes (see page 577), is a sad business. Jones, too often described as "colourful", is in reality a serious person who has done a power of good for Australian science. His consistent message in office has been that Australia, with the conviction that it must make its way in the world by being at least as smart as its competitors, has awakened both the government and substantial parts of Australian industry to the need for greater investment in research and modern industrial innovation. That too few may have listened to him is not his fault. He has also pushed hard, and sometimes successfully, for projects that will seriously enliven the Australian scene, notably the University of Sydney's stellar interferometer, due to be commissioned later in the year. It may be true that Jones managed to cut only a poor approximation to the standard figure of a politician, but that — many people would say — may have been his strength. It is unfortunate that Mr Bob Hawke, the prime minister, not himself the most polished of politicians, should have been unable to make better use of Jones's energy and enthusiasm. □



Call for further study of alleged leukaemia link

■ Wider study advised

■ Proposed mechanism doubted

London

The UK Committee on Medical Aspects of Radiation in the Environment (COMARE) has advised the Department of Health (DoH) that further study is needed of the link between childhood leukaemia in the village of Seascale, West Cumbria, and the fathers' exposure to radiation at the Sellafield nuclear reprocessing plant. The link was suggested by Martin Gardner and his colleagues from the University of Southampton in a controversial report released last month (see *Nature* 343, 679; 22 February 1990).

Immediately after its release, the Gardner report was referred for "urgent consideration" to COMARE, an independent scientific group that advises the government. COMARE is chaired by Professor Martin Bobrow of Guy's and St Thomas's Hospitals, London.

COMARE says that similar studies, looking at leukaemia clusters around other nuclear sites at Dounreay in Scotland, and Aldermaston and Burghfield in southern England, should be completed "as soon as possible". A new search is also needed for links between other British radiation workers' radiation exposure and national records of childhood cancer.

COMARE accepts the validity of the methods used by Gardner and his colleagues but notes that the conclusions are based on very few cases and do not prove a causal effect. The same conclusion was reached by epidemiologists asked to review the Gardner report by British Nuclear Fuels Limited, the site operators at Sellafield (see *Nature* 344, 283; 22 March 1990). Research is now needed to identify an underlying mechanism — before this is better understood, COMARE says, it cannot make any detailed recommendations on how best to protect radiation workers.

Gardner's team suggest, that radiation damage to the DNA of sperm cells may be to blame. But many radiation scientists are sceptical, particularly those who found no leukaemia excess among the children of the Japanese atomic bomb survivors.

An article in the latest issue of the pro-nuclear newsletter *Nuclear Issues* by John Fremlin, emeritus professor at the University of Birmingham, questions the evidence cited by Gardner to support his theory. Fremlin points out that the level of radiation used in a Japanese study that showed irradiated male mice sired offspring prone to develop cancer was far

higher than that received by workers at Sellafield. Irradiation produced a range of cancers, mostly in the lungs.

Uncovering the mechanism responsible for the 'Gardner effect' will need some basic biological research, but COMARE also wants more studies on the Sellafield workers: some epidemiologists suggest that exposure to chemicals, or the internal radiation dose (the amount of radioactivity actually concentrated in the workers' body tissues, rather than the 'external' doses measured from workers' film badges) could explain the results.

Another important issue is which of the two doses linked by Gardner to the leukaemia cases (a lifetime dose of more than 100 milliSieverts, or more than 10 mSv in the six months before conception), is the more significant. The apparent contradiction between Gardner's results and the low leukaemia incidence in the children of atom bomb survivors could be explained

NUCLEAR WASTE

Warning away future generations

Boston

ONE of the more bizarre problems surrounding the construction of the US nuclear waste repository in the salt beds at Carlsbad, New Mexico, was brought up last month at a meeting of the National Academy of Science (NAS) panel that is overseeing the project.

There it was pointed out that if federal regulations are to be strictly observed, then the site should be protected from human intruders for the next 10,000 years. But who knows what human society will be like in 10,000 years? Rip Anderson, a manager at Sandia National Laboratory who supervises WIPP's performance schedule for the Energy Department, is one of several officials who has to take this issue seriously. He says he will convene a working group next month and invite linguists and archaeologists to help. Preliminary discussion will focus on what kind of markers might still be effective far into the future.

The government has confronted this issue once before, during preliminary planning for the commercial high-level nuclear waste facility in Nevada. That effort led to a recommendation for a Stonehenge-like collection of metal slabs marked in multiple languages and pictograms. Vernon Daub, chief of the Energy Department's Repository Technology Branch at the Carlsbad site, says he worries that a

marker might attract rather than repel future visitors. In addition, he wonders how his group will manage to make any marker long-lived enough to satisfy the regulatory requirements. Daub says that his group is certain to be "conceptually" grappling with "the marker issue" for some time to come. Meanwhile, says Anderson, the group will review past civilizations' efforts to create lasting markers. "The Egyptian pyramids are a good example."

COMARE says that research must be well co-ordinated to prevent unnecessary intrusion into the lives of radiation workers and their families. The DoH is expected to take the lead in managing the programme, but COMARE will be closely involved, and the Health and Safety Executive (HSE) is expected to pay for much of the research. Although they are outside the remit of its review, COMARE also says that high leukaemia rates in the children of West Cumbrian farmers, steel and chemical workers should also be examined.

DoH and the HSE have already received a number of unsolicited research proposals since the publication of the Gardner report. A spokesman for the HSE says that these will be considered alongside others received after advertisements inviting new research proposals appear in the scientific press.

Peter Aldhous

The Department of Energy will probably have a little breathing space before it has to worry about reconstructing the pyramids. Last week, the Environmental Protection Agency proposed that the Waste Isolation Pilot Program (WIPP) be allowed to move into a five-year controlled test phase, during which the facility may ignore some regulations, including the need for permanent exclusion of humans from the site.

If adopted this autumn, the rule will allow the Department of Energy (DOE) to bring radioactive waste to the site from nuclear production facilities around the country during the test period.

The amount of waste shipped would be restricted to less than one half of a per cent of the repository's total planned capacity. It would be monitored over the five-year test period to see if radioactivity leaks from the repository or other problems occur.

Seth Shulman

ANIMAL RESEARCH

Conspiracy can't be proved

Washington

ANIMAL-WELFARE activists say that they have found no evidence to support their claim that the US government funded a University of Pennsylvania scientist to attack animal-rights efforts.

The researcher, veterinarian Adrian Morrison, was the target of an attack on 13 January 1990 by the Animal Liberation Front (ALF), an underground animal-rights group. Activists broke into his office and stole several drawers of his files, including his research grant applications and hundreds of personal letters, in search of evidence that he received federal funding for his animal-research advocacy efforts.

Morrison was targeted by the ALF "because he staunchly defends beleaguered researchers, wherever they may be", said a press release issued by People

for the Ethical Treatment of Animals (PETA), an animal-rights group that frequently releases information obtained by the ALF. Although the activists also criticized Morrison's use of cats in his own work on sleep disorders, the break-in was the first time a scientist had been targeted primarily for his support of animal research.

"ALF removed documents that will help establish the deep-seated corruption of the anti-animal Nazis", PETA co-founder Alex Pacheco promised last month in *PETA News*, the organization's magazine. But in the months since the break-in, no evidence has surfaced to support the claim that Morrison was being surreptitiously supported by the US Public Health Service.

Animal-rights activists have pointed out that a memorandum written in 1987 by Frederick Goodwin, then director of intramural research at the National Institute of Mental Health, called for a more "pro-active" opposition to the animal-rights movement. One of Goodwin's suggestions was "to fund special fellowships in research advocacy for investigators who may wish to include a year or two of such activity in their career". Morrison was seen by animal-rights activists as the first of such federally funded animal-research advocates.

Mary Beth Sweetland, a PETA investigator who has been analysing the stolen files, said last week that "I don't think that anything in the files will provide absolute proof of what we believe to be a binding link between NIH [National Institutes of Health] and Morrison".

Goodwin, who is now the director of the Alcohol, Drug and Mental Health Administration, the sister agency to the NIH, says Morrison was funded for research alone. "Everything he's done — and I think he's done a lot of courageous things — he's done on his own", says Goodwin.

Morrison has been on sabbatical leave since the attack. He says his advocacy efforts were motivated by his concern about the tactics of animal-rights groups. "The claims of the animal-rights extremists against biomedical research and individual researchers [have] been so distorted and vicious, and the threat to the public's health so great, that I could not stand by quietly", he says.

His stolen files do show that Morrison, who is chairman of the Society of Neuroscience's Committee on Animals in Research, wrote hundreds of letters (319 in 1989 alone) in support of animal research, testified in court and gave dozens of lectures in defence of animal research and animal researchers.

G. Christopher Anderson

AUSTRALIAN RESEARCH

Ministerial time up for Mr Jones

Sydney

BARRY JONES, the colourful and controversial science minister who believes he raised Australia from being 'the lucky country' to being 'the clever country', has lost the post he has held for seven years as Minister of Science and Technology following the recent federal election.

Jones has been replaced by Simon Crean, an avowed 'economic rationalist', whose additional role as Minister Assisting the Treasurer may deflect his attention away from science and technology. Crean has been brought into the cabinet as new blood and is a strong candidate to take over from the present Treasurer, Paul Keating, who seems certain to be elected leader of the Australian Labor Party.



Barry Jones — replaced by 'new blood'.

In a protest over his dismissal, Jones has threatened to resign from Parliament and force a risky by-election. The Labor government won the 24 March election by the small margin of 78 seats to 69 for the opposition. The government is thought to be trying to persuade Jones to remain in Parliament by offering him a position such as ambassador to UNESCO. Sources within Parliament say he has refused.

Jones was elected in 1972 and appointed Opposition spokesperson on science and technology in 1980, becoming minister following the election of the Hawke government in 1983.

Jones told the *Sydney Morning Herald*: "Often when my ideas have been first proposed they were derided. Then, on the second time around, they are roundly applauded and embraced, but never connected with me." It has long been felt, even by his allies, that Jones was politically naive. In a highly factionalized party, he failed to develop a strong political base.

It was partially scientists' vocal dissatisfaction with funding cuts that raised the profile of Jones and his ministry. As late as last year, the Prime Minister acknowledged the vote-catching potential of science by providing increased funding. Jones's policy of "making science pay" is likely to be continued by the new minister.

Tania Ewing

GLOBAL CHANGE

No surprises in leaked report

London

A PRELIMINARY version of the report from the science working group of the Intergovernmental Panel on Climate Change (IPCC), which is due to be made public in late May, has been leaked to Independent Television News in the United Kingdom. The IPCC scientists predict that by the middle of the next century, global temperatures will be an average 3 °C higher than today, with average rainfall some 7 per cent higher. Sea level may rise by about 45 cm. But there will be local variations, the IPCC predicts, with greater warming in the northern hemisphere, and regional droughts and flooding.

A similar view has been reached elsewhere but the report is important in the influence it is expected to have on official policy throughout the world. The report comes from one of three working groups within IPCC (the others are examining the impacts of climate change and policy to combat the problem).

About 200 scientists, under the chairmanship of John Houghton of the UK Meteorological Office, were involved in the working group, and met at a series of workshops over the past year.

Geoff Jenkins, a member of the working group, also at the Meteorological Office, says that the report has been sent out to more than 100 scientists for peer review. Given the large number of people involved in compiling and reviewing the report, he thinks it was almost inevitable that the report's findings would be leaked in advance of the publication date.

Peter Aldhous

Sun sets on launch monopoly

Tokyo

DRASTIC reorganization is on the way for the National Space Development Agency (NASDA), the larger of Japan's two space organizations, after Japan last week gave in to US demands for greater access to its satellite market.

The dispute centred on a new generation of communication satellites (CS-4) that NASDA planned to develop for launch in the mid-1990s with substantial financial support from Nippon Telephone and Telegraph (NTT), Japan's partly privatized domestic telecommunications organization. Two CS-4 satellites were to be launched and used by NTT.

The United States argued that CS-4 is a commercial project and should be open to foreign bidding. Japan countered with the view that it is a non-commercial research and development project.

But last week, Japanese negotiators in Washington agreed to revise the CS-4 project completely. NTT will no longer participate and is free to buy foreign-made telecommunications satellites (something the company has long wanted to do because US satellites are much cheaper than those made in Japan). Only one CS-4 satellite will be built by NASDA and its development will be merged with that of

an Experimental Data Relay and Tracking Satellite (EDRTS) to make the project less commercial in nature.

The agreement affects much more than just the CS-4 project. Japan has agreed that all government-procured commercial satellites, including broadcasting and meteorological satellites, will now be open to foreign bidding and NASDA will be restricted to the production and launch of research satellites. Fifty per cent of the satellite projects planned by NASDA will now be abandoned, including a geostationary meteorological satellite (GMS-5) and a new generation of broadcasting satellites (BS-4), according to Katsuo Yonezawa of NASDA's international division.

Although loss of these satellites could cut drastically into NASDA's budget, Yonezawa says that more money will probably be directed into NASDA's participation in the US space station project and "other new research projects". Agency officials are also worried about loss of payload for the new H-2 rocket which is currently scheduled for first launch in 1993. The satellites that will have to be abandoned by NASDA would have been among the first to be launched by the H-2.

NASDA officials are hoping that the H-2 will be used to launch some US scientific payloads instead. An agreement has already been reached with the US National Aeronautics and Space Administration (NASA) to launch a US satellite for monitoring tropical rainfall (TRMM) with the H-2 in 1996. The H-2 may also be used to launch some of a series of US life science satellites (LifeSat) in the mid-1990s.

Last week's agreement will require revision of Japan's space policy guidelines, according to Yonezawa. He says that it is not yet clear who will be responsible for the launch of commercial satellites such as broadcasting and meteorological satellites although they may go to the European Space Agency's Ariane launch vehicle or other foreign commercial launch vehicles.

With NASDA responsible for only research satellites, the distinction between NASDA and its smaller brother, the Institute of Space and Astronautical Science (ISAS), which launches scientific payloads, will blur. But as the two space agencies are attached to different government organizations — NASDA to the Science and Technology Agency (STA) and ISAS to the Ministry of Education, Science and Culture (MESC) — it is unlikely that the two will be merged. Instead, NASDA will probably concentrate on developing manned space activities while ISAS will focus on small scientific interplanetary missions.

David Swlnbanks

Rocket soars as science is attacked

Tel Aviv

As *Offeq II*, Israel's second satellite, was launched into space on an Israeli rocket last week, the country's civil service commissioner called for the closure of the Ministry of Science and Technology, along with four others, as a "money-saving measure".

Meir Gabai, who is responsible for Israel's 45,000 civil servants, said there was "no justification" for the large number of ministries (22) in Israel. He pointed out that Switzerland has just seven ministries. But Arye Shumer, director-general of the Ministry of Science and Technology, retorted that unless the Science Ministry "becomes one of the five ministries with the highest priority and budget, science will continue to decline". Shumer attacked the Treasury for not allocating enough to science in the latest budget, finalized in the Knesset earlier this month. But he also said that the \$600 million budget for civilian and military research and development would be "almost enough" if one body, "preferably the Science Ministry", could coordinate its distribution. Shumer also criticized "the 15 professors who sit in the Knesset" for not doing enough to advance the cause of science.

One of those professors, Yuval Ne'eman, chairman of the Israel Space Agency, spent last week denying reports that *Offeq II* is a spy satellite. He insists that its mission is purely scientific. The 160-kg satellite has the same dimensions as its predecessor (launched in September 1988) but has a larger memory and the capability of two-way communication. Both were launched with a solid-fuel Shavit rocket, which Ne'eman has compared with the Chinese Long March or the Vostok SA3, but *Offeq II* is expected to have a shorter lifespan than the four months of *Offeq I*. The next in the series, *Offeq III*, will be launched in 1992.

Lisa Perlman

Smaller greenhouse satellites?

Washington

NASA (the National Aeronautics and Space Administration)'s plan to launch two large satellites at the end of the decade to monitor the impact of the greenhouse effect may miss key measurements, the National Academy of Sciences said last week in a preliminary review of the US global change research programme.

Several smaller satellites could make simultaneous measurements at different altitudes and positions, allowing researchers to observe the climate as a whole, the academy points out. A single large satellite makes it necessary for all the onboard instruments to fly in the same orbit and increases the risk that a sensor failure could result in long gaps between measurements. Soil moisture and global biomass may also not be adequately measured because the only instrument that can monitor them from space — synthetic aperture radar (SAR) — is not included in the NASA Earth Observing System (EOS) plans, the academy said. Because SAR instruments are not compatible with the planned EOS satellites, the academy recommends a free-flying SAR mission.

G. Christopher Anderson

Pegasus flies

Washington

PEGASUS, the first privately developed US rocket to carry a payload into space, was successfully launched from a B-52 bomber last week. The 10-minute flight carried into orbit a Navy communications satellite and a 422-pound National Aeronautics and Space Administration (NASA) payload that measured launch vehicle conditions. At a cost of \$6.5 million a launch, the flight marks a promising beginning for the US effort to find a cheap and reliable way of sending small satellites into space. Because it is launched by an aircraft, the 50-foot winged rocket can put payloads into orbit at a cost of about \$6,000 a pound, compared with about \$20,000 a pound for ground-launched rockets.

G. Christopher Anderson

Laser fusion misses targets

Washington

LASER-DRIVEN fusion research has not progressed as quickly as hoped because of technical difficulties, funding shortfalls and problems with one small private contractor, the US Department of Energy (DOE) recently told federal investigators.

As part of an investigation completed last month by the US General Accounting Office (GAO), DOE officials revealed that the US inertial-confinement fusion experiments have turned out to be more difficult than anticipated, putting parts of the programme as much as two years behind schedule. The officials blame many of the delays on "unsatisfactory" performance by KMS Fusion, a Michigan-based private contractor that made parts for the laboratories.

DOE officials also said that KMS lobbied Congress to give it more money than DOE had requested, thus leaving less for other US fusion laboratories. Almost all the DOE laboratories working on inertial-confinement fusion have slipped behind schedule, GAO says. For example, Los Alamos National Laboratory completed only about half of its 1987-89 objectives on time, because its experiments turned out to be unexpectedly complicated and because KMS was late in delivering target components. Sandia National Laboratory, which is researching particle-beam-driven fusion, is one to two years behind schedule because of difficulty in developing a source of lithium ions for its particle beam. Sandia officials also blame KMS for a congressionally mandated \$1.9 million reduction in their budget last year. Congress shifted a total of \$3.1 million from the DOE labs to KMS in 1989 — a year

after the company had donated nearly \$17,000 to the re-election campaigns of 18 representatives.

According to GAO, its investigation was precipitated by DOE's complaint that KMS had "not met program expectations". Largely because the agency had asked it to abandon much of its own research in favour of supporting efforts at the national laboratories, KMS had trouble "transitioning", GAO found. "Everyone else in the program gets to set their own objectives. We got ours set by the national labs," says KMS president Patrick Long. The DOE national laboratories claim that KMS is "adept at winning additional funding recommendations from the Congress at the expense of the DOE laboratories", GAO reports. "This has led to complaints from the DOE national laboratories that some of their experiments had to be delayed and objectives postponed." DOE programme director Sheldon Kahalis declined to comment on the report.

HUMAN FRONTIERS PROGRAMME

Grant winners announced

Tokyo & London

TWENTY-NINE international teams of scientists were last week awarded the first grants for research on the brain and molecular biology under Japan's Human Frontier Science Programme. The successful teams were chosen from more than 200 applications. A greater number of applications is expected next year and the programme's budget will have to be increased drastically to maintain even the present level of awards.

The grants awarded last week range from \$150,000 to \$400,000 a year and are to run for three years. Teams led by Japanese won the most grants (9), followed by teams headed by US (8), British (5) and French (3) scientists. The distribution of nationalities fairly closely reflects the number of applicants from each country (see *Nature* 343, 684; 1990) and should help dispel any doubts that the screening process is fair (see *Nature* 344, 479; 1990).

One of the two biggest grants of \$400,000 a year goes to a team from the United States, West Germany and Japan led by Fuyuhiko Inagaki of Tokyo Metropolitan Institute of Medical Science. The team will try to elucidate the molecular mechanisms of hormone-receptor interaction and signal transduction. The second \$400,000 grant goes to a United States-Japan team led by Yasuhiro Anraku of Tokyo University which will investigate the molecular mechanics of electron transport-coupled phosphoryla-

In the past decade, the KMS political action committee has contributed nearly \$64,000 to congressional election campaigns, according to the Federal Election Commission, an unusually active lobbying effort for such a small laboratory. But as a private company, "that is our prerogative", says Long. He also points out that KMS invested \$30 million in research that led to a patent for inertial-confinement fusion. Citing laws that allow the government to annex technology for national security, "DOE took a royalty-free licence", Long says. Any congressional largesse reflects a sense that the company is rightfully entitled to proceeds from the invention, he says. "What kind of annual return should you get on an investment of \$30 million? The most KMS has ever earned is \$1.2 million [a year]." Although KMS has performed better recently, DOE plans to open the competition for the KMS contract to other companies later this year. In its 1991 budget proposal, DOE has cut its request for KMS by 45 per cent to \$10 million, and asked the company to concentrate only on target fabrication.

G. Christopher Anderson

ROYAL SOCIETY

Atiyah for president

London

THE Royal Society's Council has chosen Sir Michael Atiyah, a mathematical physicist, as its preferred candidate to replace Sir George Porter as the society's president



from 30 November. In the past, the council's candidate has always later won the approval of the fellows who choose the president in an election. Atiyah is a Royal Society research professor at the University of Oxford, and president-elect of Trinity College, Cambridge. Peter Aldhous

tion. A team from Sweden and France led by Jacques Mallet of CNRS receives \$385,000 to examine genetically-engineered cells producing neurotransmitters.

But while the grant money goes to a wide selection of countries, funds for Frontier postdoctoral fellowships will end up largely in the United States. More than half (45) of the 80 successful fellowship applicants have chosen to go there. Twenty-two will go to European countries, and only three to Japan.

A total of \$12 million has been spent on this year's awards, with Japan providing \$11 million and France \$1 million. Frontier organizers expect more applications next year and some money from this year's Japanese budget of ¥2,400 million (\$15 million) will be held over for next year.

Italy, whose scientists did not fare very well in last week's awards, will contribute 1,000 million lira (\$0.8 million) next year and some of the money will be used to publicize the programme in Italy. Switzerland, Sweden and Australia have offered to contribute small sums if they are allowed to join (a decision on membership of non-summit nations is expected this month). Japan's budget for the programme in 1990 has been increased 34 per cent over last year to ¥3,200 million (\$20 million). If the number of awards is to be increased to cope with the anticipated increase in number of applications the programme will need more funds to cope.

David Swinbanks & Peter Aldhous

US medical research healthy

London

A RECENT report suggesting that US clinical research is in decline because of shortfalls in funding is misleading, according to the editors of leading medical journals.

In the 15 March issue of the *New England Journal of Medicine* (322, 739–742; 1990), Thomas and Sage Stossel, from the Massachusetts General Hospital, show that original research papers with at least one US author became progressively scarcer between 1978 and 1988. But clinical research in other parts of the world has been on the rise (see figure). Although reluctant to draw firm conclusions from this, the researchers suggest that the drop “coincides with the slowed growth of funding from the National Institutes of Health for US clinical research”.

But editors think otherwise. Stephen Lock, editor of the *British Medical Journal* (BMJ), says that the report is “simplistic” because it does not account for subtle shifts in editorial direction that may influence a researcher’s choice of journal. Lock’s impression is that US research is as healthy as ever, but each journal presents a special case. For example, the fact that the *BMJ* is British “puts Americans off”. Not that this deters researchers from Japan or continental Europe, in particular Scandinavia. So US appearances in the *BMJ*, at least, may be more a function of national attitude than research acumen.

Gordon Reeves, editor of the *Lancet*, agrees: like Lock, he highlights Scandinavia as a brightening focus of excellent medical research, but says to suggest a decline in US research would be misleading.

According to the report, published papers with no US input have maintained a steady presence in the *Lancet* for the past decade, whereas US papers have become fewer, declining from a peak of 139 in 1980 to only 32 in 1987. But Reeves

expects a revival of US research papers in the *Lancet* following an increase in US subscriptions and the establishment, this year, of a US office close to the National Institutes of Health in Maryland.

Even if US medical research is really on the slide, says Lock, the causes may be more complex than a simple decline in funding. German medical research was paramount before the Second World War, he says, and Viennese work before that. The quality of Japanese and European research is in the ascendant for a variety of reasons, says Lock, and US research could be past its peak. But for the moment, at least, reports of its imminent demise have been exaggerated.

David Pendlebury of the Philadelphia-based Institute for Scientific Information (ISI) and editor of *Science Watch* is concerned about the choice of journals sampled by the Stossels. He questions the inclusion of the British-dominated

INTERNATIONAL COLLABORATION

No to fortress Europe

London

THE Royal Society has warned against adopting a ‘fortress Europe’ attitude towards Britain’s future international collaborations in science. Professor Anthony Epstein, the society’s vice-president, told the House of Commons Education, Science and Arts Committee that Britain should foster scientific contacts with East European countries, and not concentrate just on projects within the European Communities (EC).

World science has three ‘gravitational centres’ — the United States, Japan and Europe — Epstein said, and if Europe is to compete, Eastern European manpower and expertise should be drawn upon.

The EC’s funding of science through its Framework programme may, in any case, be flawed, according to Epstein. He said the programme suffered through its rigid direction from the European Commission and strong bias towards applied research.

The lack of adequate peer review, Epstein suggested, may also mean that Framework-funded projects are inferior to those supported at the national level. The society’s written evidence to the committee nominates the Strasbourg-based European Science Foundation (an association of European research councils and academies) as a candidate for the peer-review task, with Academia Europea providing further advice. But Epstein said that this would need a substantial diversion of EC funds to the two bodies.

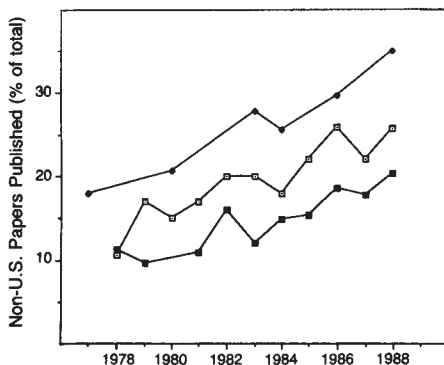
Peer review typically takes up only 1 per cent of a science budget, Epstein said, but

Lancet, and regards the omission of *Journal of the American Medical Association* (JAMA), as a major flaw. In a survey of the ISI’s databases, carried out for *Nature*, and reviewing a wider selection of medical journals, Pendlebury finds little evidence for a marked decline in publications featuring a US author over the 1980s (although he stresses that his own survey includes a wider range of contributions than original research articles alone).

Pendlebury does note a decline in the percentage of papers in the *JAMA* with US authors, from 84.3 per cent in 1981 to 64.2 per cent in 1989, mostly due to a decline in the early 1980s. But he says it is difficult to interpret this trend.

George Lundberg, editor of the *JAMA*, says the American Medical Association says that preliminary results of a survey of papers submitted to, and published in, its journals suggest that the number of US submissions and their acceptance rate have been “pretty stable” over the last several years.

Henry Gee & Peter Aldhous



Non-US papers published by the *New England Journal of Medicine* (open squares), the *Journal of Clinical Investigation* (solid squares) and *Blood* (diamonds), 1977–88. (Reprinted by permission of the *New England Journal of Medicine* 322, 739; 1990).

in the context of the Framework programme’s five-year budget of 5,400 million ECU, this is a large sum.

British national science policy also limits the effectiveness of British scientists’ involvement in EC-funded science, according to the society’s witnesses. Sir Eric Ash, rector of Imperial College, London, said that, unlike institutions in many other EC countries, British universities receive no funds to cover overheads arising from EC projects. Ash likened the effects of accepting an EC grant in financially squeezed British universities to “drinking seawater when dying of thirst”. Epstein added that financial stringency meant that British universities often have to charge ‘bench fees’ to visiting scientists from abroad.

Not surprisingly, Epstein pointed to the Royal Society’s own exchange schemes as a role model for international cooperation. The society is a small-money player in international terms, but spends nearly 30 per cent of its annual budget from the Department of Education and Science on international collaboration.

Epstein explained that the Hungarian Academy of Sciences is to join the Royal Society’s European Science Exchange Programme, and discussions are under way with the Polish Academy. He stressed the importance of the society’s concentration on exchanges at the postdoctoral level. Britain should promote exchanges with East European scientists in their formative years, Epstein said, not visits from “70 year-old academicians”.

Peter Aldhous

NUCLEAR-POWERED SHIPS

Mutsu's last chance

Tokyo

JAPAN's one and only nuclear powered ship, the infamous *Mutsu*, has sprung to life after sitting rusting in port for 16 years. The ship, which has gobbled up hundreds of millions of dollars in public funds, will be given one last chance to prove itself before being mothballed next year. The saga of the *Mutsu* draws attention to the lack of a central organization to coordinate and monitor the activities of the various science-related ministries and agencies in Japan.

Mutsu was launched in 1968 and was ready for sea trials by 1972. But local fishermen, fearing radioactive pollution from the ship, prevented *Mutsu* from putting to sea from Mutsu City port in northern Japan for two years. When the ship finally set sail, disaster struck almost immediately. The reactor began to leak and the crew had to stuff rice and socks into it to try and stem the flow of radiation as the ship headed back to home port where local fishermen barred re-entry until suitable compensation was paid and the government agreed to dock the ship elsewhere (see *Nature* 310, 531; 1984).

Mutsu then wandered from port to port (never under its own power) in search of a home. Fourteen years later the ship eventually found its way back to Mutsu City where it has been anchored at a custom-built new port for the past two years.

Last week, the reactor was started up and reached criticality. The Science and Technology Agency (STA), which is responsible for the ship, had planned to start up the reactor last year but some of the

fuel rods and control rods were found to be rusty after being idle for 16 years and had to be replaced.

If all goes well with the test runs, *Mutsu* will set sail in about five months on a final year-long cruise to the South Pacific (provided fishermen and anti-nuclear activists do not intervene). But some experts have expressed fears that extra concrete and zirconium shielding that have been added around the reactor may have made the ship top heavy and unstable in rough weather. It also seems unlikely that much will be learned from the tests. Western nations gave up the idea of commercial nuclear-powered ships decades ago. And Japan has no plans to follow up *Mutsu*. The main argument that STA officials offer for carrying out the tests is that they have to collect data to justify spending ¥117,000 million (\$740 million) over the past three decades.

Such an attitude illustrates a major defect in the Japanese government's management of science and technology projects. Once big projects get under way, there is no mechanism or central organization (such as the Office of Technology Assessment in the United States, for example) to re-appraise them or change direction. And government ministries and agencies determined to maintain their own territory and budgets will keep pumping money into projects no matter how absurd and out of touch they may be.

The final fate of *Mutsu* is unknown. It may be made into a museum; another possibility is that it will be converted to diesel power.

David Swinbanks

INTERNATIONAL ORGANIZATIONS

United States says 'No' to UNESCO

Washington

DESPITE increasing pressure from scientists for the United States to rejoin the United Nations Educational, Scientific and Cultural Organization (UNESCO), the State Department last week said that it would not do so until the organization is better managed and politically unbiased. Recent reforms by UNESCO director-general Federico Mayor have caused internal strife in the organization, and his appointment of a Soviet citizen to direct communications and information has been opposed (see *Nature* 344, 368; 29 March 1990). "Very little has changed since the United States withdrew at the end of 1984", said State Department spokeswoman Margaret Tutwiler. She said that a full explanation for the decision will be submitted to Congress on 17 April.

G. Christopher Anderson

UK RESEARCH COUNCILS

No news is bad news for appointments to advisory board

London

The UK Department of Education and Science (DES) is finding the appointment of independent members to the reconstituted Advisory Board for the Research Councils (ABRC) more time-consuming than expected.

When Secretary of State John MacGregor announced on 19 January that the ABRC would be given a more explicit remit to improve coordination between the research councils, with its membership cut from 26 to 14 (see *Nature* 343, 294; 1990), he hoped that the board's full membership would be announced "within a few weeks".

The new arrangements were to take effect from 1 April, but as *Nature* went to press, Peter Thorpe, secretary to the ABRC, was still waiting for an announcement from the DES. "We have been pressing for the appointments to be made quickly", he says, and adds that he "didn't assume we would be into April" before the final shape of the board was made certain. The reasons for the delay are unclear, but a spokesman for the DES says that an announcement is likely to be made "very soon".

The new ABRC will be chaired, as before, by Sir David Phillips, of the University of Oxford, and will comprise the five heads of the UK research councils, two assessors (a representative from the DES and the Chief Scientific Adviser from the Cabinet Office) and six independent members. Time will tell if the new-format ABRC can improve coordination between the research councils, a matter that MacGregor has asserted demands "urgent attention".

The most pressing concerns are the conflicting claims for primacy in biotechnological research made by the Science and Engineering Research Council and the Medical Research Council, and for better coordination between the land-based environmental research programmes of the Agricultural and Food Research Council and the Natural Environment Research Council.

One worry is that in remaining merely as an advisory body to the DES, the ABRC will lack the power to take its proposed "interventionist" role in disputes between research councils (see *Nature* 343, 293; 1990). Two members of the old ABRC, both government department chief scientists, argued that the new body should be given stronger executive powers, but were defeated by a majority ABRC decision. The first meeting of the new ABRC is planned for 20 April, but Thorpe says that the DES should move quickly if the independent members of the board are all to attend.

Peter Aldhous

Appointments still under tight control

Cape Town

THE South African government last week refused to grant a work permit to the vice-rector-designate of the University of the Western Cape (UWC), Professor Njabulo Ndebele. Professor Ndebele, 42, is the national president of the Congress of South African Writers, and at present holds the position of deputy vice-chancellor of the National University of Lesotho. He is a citizen of that country.

Reacting to the announcement, the rector of UWC, Professor Jakes Gerwel, said: "Professor Ndebele is one of the most highly esteemed intellectuals in southern Africa, and we were really not anticipating that in the present climate a person of his stature would be refused a work permit". Gerwel has asked for an urgent meeting with the Minister of Home Affairs, Gene Louw.

Michael Cherry

Women winners of Nobel Prize

SIR—In my review of *Grand Obsession: Marie Curie and Her World* (*Nature* **343**, 707; 1990), I said that Marie Curie was the only woman to have won the Nobel prize for physics. I should have known better, and now I do — thanks to all those who have written to me. Maria Goeppert-Mayer won the prize in 1963 for the shell theory of the atomic nucleus.

JOHN GALLOWAY

*Cancer Research Campaign,
2 Carlton House Terrace,
London SW1Y 5AR, UK*

SIR—John Galloway's book review needs some correction.

(1) Marie Curie is not "the only woman ever to have won the prize for physics". Maria Goeppert-Mayer won the 1963 Nobel physics prize.

(2) "No woman other than a Curie had won the prize for science until Dorothy Hodgkin in 1964" is also erroneous. Apart from Mayer, Gerty Cori won the Nobel prize for medicine in 1947 with her husband Carl Cori.

(3) So far, there have been 21 women Nobel prizewinners:

Chemistry — Marie Curie, Irene Joliot Curie and Dorothy Hodgkin; Physics — Marie Curie and Maria Goeppert-Mayer; Medicine — Gerty Cori, Rosalyn Yalow, Barbara McClintock, Rita Levi Montalcini and Gertrude Elion; Literature — Grazia Deledda, Sigrid Undset, Pearl Buck, Gabriela Mistral and Nelly Sachs; Peace — Bertha von Suttner, Jane Addams, Emily Balch, Mairead Corrigan, Elizabeth Williams, Mother Teresa and Alva Myrdal.

SACHI SRI KANTHA

*Department of Physiology
and Biochemistry,
Medical College of Pennsylvania,
Philadelphia, Pennsylvania 19129, USA*

Material increases

SIR—Under the heading "Hidden increases", Ian Winship (*Nature* **342**, 730; 1989) complained about publishers adding new sections to an established periodical and the consequent increase in price. In the case of *Journal of Materials Science*, which he mentions, there was nothing hidden; information about the changes was circulated at the usual time in the preceding autumn.

Our reason for adding the new sections was that research in materials science is burgeoning, and that the increasing number of good papers being submitted for publication would soon have led to a serious backlog. We did not want to split the journal because its point is that it

covers the whole field. And it would in any case have been difficult to transfer papers destined for publication in the journal to new, more specialized ones, because of the problem of building up subscriptions from scratch: our evidence was that contributors would simply refuse.

That is why we started two quarterly adjunct journals in key growth areas (materials in medicine and materials in electronics), both of which are part of the main subscription and available separately for those with specialist interests. That had to be reflected in an increased subscription rate, although we did not in practice pass on all the extra costs to subscribers.

Like other librarians faced with the need to manage budgets that are declining in real terms, Winship blames publishers for increasing the size and price of journals. He forgets that, to survive, publishers have to be responsive to the needs of the scientific community, which in some subjects means providing supplementary journals for the increasing number of good papers that are offered.

ANTHONY WATKINSON

*Chapman & Hall,
11 New Fetter Lane,
London EC4P 4EE, UK*

Random chaos

SIR—Thomas Ochs¹ asks too much when he requires authors to be as stringent in the use of 'random' as in the use of 'chaotic'. It is impossible to prove that something is truly random. For any system, it is always possible that a discovery will lead to predictability, even if this is only partial. For many systems, that of tossing an unbiased coin being one, it is only the limits of observation that prevent prediction. The only logical conclusion of Ochs's argument is that random must, in all its uses, be preceded by 'apparently' or prefixed by 'pseudo'.

What puzzles me is why the word 'chaos' was chosen. The dictionary meaning is 'utter confusion'², which dynamic chaos plainly is not. Explaining chaos to those who know nothing about it is not made easier by such a commonplace word. At the least, spelling it 'kaos' would have helped but even better would have been a word from a nonsense poem, or something similar. The physicists found 'quark' when naming subatomic particles; it is too late now, but 'caucus' might have done instead of 'chaos', from Lewis Carroll's caucus-race. This required a race-course in a sort of circle whose exact shape did not matter; the competitors were placed "along the course, here and there";

Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only. □

and there was no start or finish, so it was not easy to tell when the race was over. The connection between caucus, politics³ and what we now term chaotic behaviour would have added piquancy.

NEVILLE W. GOODMAN

*University of Bristol,
Department of Anaesthetics,
Southmead Hospital,
Southmead Road, Bristol BS10 5NB, UK*

1. Ochs, T.L. *Nature* **343**, 303 (1990).

2. *Concise Oxford Dictionary*, 7th Edn (1982).

3. Gardner, M. (Ed.) *The Annotated Lewis Carroll* (Harmondsworth, Penguin, 1970).

Problems

SIR—I am less inclined than is J.Z. Young to be grateful for the latest lucubrations of Sir John Eccles, or to feel that they deal with "serious and difficult problems of science and philosophy" ("A change of mind", review of *Evolution of the Brain: Creation of the Self*, *Nature* **344**, 117; 1990).

They deal with Eccles's religious inclinations, for example: "... I am constrained to attribute the uniqueness of the Self or Soul to a supernatural spiritual creation ... each Soul is a new Divine creation which is implanted into the growing fetus at some time between conception and birth". Parliament, now exercising itself over a similar conundrum, would doubtless be interested to know exactly when this miracle occurs. At 14 weeks? 18 weeks? 22, 24, less than 14, more than 24? Can Eccles be a little more specific?

And does he believe, as Young indicates he does, that all mammals and birds have 'souls'? But not reptiles, amphibia, fish, bees and cephalopods?

Young asks at what stage of human evolution did hominids first receive their souls. The matter is in fact covered by the 1950 papal encyclical *Humani Generis*, which explains that the soul was acquired during the early Pleistocene, 800,000 years ago, apparently in Kenya.

While Eccles's meditations on these "serious and difficult problems", at £30 a meditation, are no doubt of interest to Eccles, they are of no concern to anyone else.

I have, as you may recall, brought matters of this kind to your urgent attention on two previous occasions (*Nature* **323**, 754; 1986 and **338**, 536; 1989). Questions of one's religious convictions do not concern Me but their espousal within these pages does, this because, although certainly not a jealous God, I am a serious-minded One. Accordingly, I do not feel they form a necessary adjunct to scientific methods or attitudes.

GOD

(As revealed to Ralph Estling)

*The Old Parsonage,
Dowlsh Lake,
Ilminster, Somerset TA19 0NY*

Where prediction is not possible

For those — such as Californians — who live at plate boundaries, earthquakes are a fact of life. Others, who may count themselves lucky, should think again.

London

READERS elsewhere may be forgiven for not knowing that last week Britain had what may have been its biggest earthquake this century. No one was injured, and the reported structural damage was limited to ruptured gas mains, cracked masonry and collapsed chimneys. With a magnitude of about 5, the event on the Anglo-Welsh border was only one-hundredth the size of last October's earthquake on the San Andreas fault; indeed, California has a few earthquakes this size each year.

The difference is that the San Andreas fault marks the boundary between two crustal plates, whereas Britain sits well within the borders of a plate that stretches from the Mid-Atlantic Ridge to Japan, and from the Arctic Ocean to the Mediterranean. The plate boundaries are where almost all the action is: plot the world's earthquakes and active volcanoes on a map and the plates emerge, as in a game of connect-the-dots.

But what of the stray dots in the middle — the intraplate earthquakes? They signal the failure of the simplifying assumption of plate tectonics, that the plates are rigid monoliths which deform only at their edges. And whereas plate tectonics provides a framework in which to understand — and perhaps ultimately to predict — plate-boundary earthquakes, there is as yet no analogous theory for earthquakes in 'stable' continental crust. No seismologist can say, even after the fact, why last week's earthquake occurred where or when it did, or why it was the size it was.

Of course, these questions cannot be answered with certainty for any earthquake, but at least at plate boundaries one can see (and measure) the direct cause of the activity. For example, the Pacific plate is moving northwards with respect to North America at about five centimetres a year. If, instead of sliding smoothly by one another, the two plates are locked together by friction on the fault, then stress will accumulate at the boundary. When the fault does slip, it does so catastrophically, in an earthquake whose size depends, in part, on the amount of slip.

Experience with the San Andreas has led to the definition of several fault segments with different behaviour: some creep aseismically; others slip frequently in small earthquakes; still others seem to store up energy for longer times and rupture in great earthquakes. Although this idea of 'characteristic earthquakes' is

not universally accepted (and may not apply to all faults), at least one can say how much of a 'slip deficit' is likely to have accumulated at various parts of a plate-boundary fault, and put an upper limit on the size of earthquake that may occur.

Away from plate boundaries, life for seismologists is more difficult. Most intraplate earthquakes cannot be assigned to an identifiable fault zone, in part because most are too small to rupture the Earth's surface, but also because the low level of intraplate seismicity makes it difficult to identify 'active' zones. Most continental interiors are characterized by a rather uniform state of stress, arising from forces acting on the edges and base of the plate (see M. L. Zoback *et al.* *Nature* **341**, 291–298; 1989). Typically, the deformation rate that results is only one-thousandth of that at a plate boundary such as the San Andreas; to generate large earthquakes the strain must somehow be concentrated.

Arch Johnston, of Memphis State University, has pointed out that the largest intraplate earthquakes are associated with areas of continental crust that have been weakened by an episode of stretching and thinning, during the formation of a rift or a new ocean basin. (This is bad news for the heavily populated eastern seaboard of the United States, which was stretched during the early stages of the opening of the Atlantic Ocean.) But the correlation breaks down at smaller, yet still damaging, magnitudes; notably, some of Australia's largest onshore earthquakes have occurred in very old, unrifted crust.

If all intraplate earthquakes were as benign as last week's event in Britain, the failure to understand them would represent an intellectual challenge and no more. But historical records show that past intraplate earthquakes have been as powerful as the great San Francisco earthquake of 1906. In New Madrid, Missouri, three magnitude 8 earthquakes occurred in a three-month period in 1811–12, damaging structures 1,500 km away on the Atlantic seaboard. And, given modern population densities, even a modest earthquake in an urban area can be devastating: last December's magnitude 5.5 event in Newcastle, Australia, killed 12 people and caused A\$1,500 million worth of damage.

Seismologists are fond of saying, "Earthquakes don't kill people; buildings do". The long gaps between large intraplate earthquakes — for example, 500–1,000

years for the recurrence of a magnitude 7 earthquake on the New Madrid zone — have bred complacency about seismic protection east of the Rocky Mountains. In fact, residents of the eastern United States are only now beginning to realize that their seismic risk (a term that takes into account not just intrinsic earthquake hazard, but factors such as population concentration, soil conditions and building codes) is comparable with that of Californians. As a result, Memphis (the largest city near the New Madrid zone) has just adopted seismic resistance requirements, and New York City is about to follow suit.

The buzz of regulatory activity may give the impression that the intraplate earthquake problem is in hand, at least in principle; that what we lack in predictive skills can be made up for by engineering. But there is a worrying loose end, which calls into question the ability to estimate the seismic hazard of an intraplate region: intraplate seismicity seems to be inherently patchy in time and space, and more so than can be explained simply by the statistics of small numbers.

A particularly striking example, only recently recognized, is the Meers fault in southwestern Oklahoma. A conspicuous fault scarp, 30 km long, bears witness to a faulting event about 1,200 years ago (A. J. Crone & K. V. Luza *Geol. Soc. Am. Bull.* **102**, 1–17; 1989). If the event was accompanied by an earthquake (which must be confirmed by showing that the fault extends downwards several kilometres into the crust), the earthquake would have had a magnitude of about 7. Yet today, unlike the New Madrid zone, the area surrounding the Meers fault is essentially aseismic. In the New Madrid zone, the likelihood of a major earthquake is estimated by extrapolation from the frequency of smaller earthquakes, but how does one estimate the seismic hazard of the Meers fault?

A further obstacle to understanding is thrown up by the observation that, before the event 1,200 years ago, the Meers fault had not moved for at least 100,000 years. If recurrence times can be as long as 100,000 years (and if there can be no seismicity in the meantime), there must be areas of the continents capable of significant seismic activity about which we know nothing at all. Frustrating for seismologists, perhaps, but there should be plenty of work for structural engineers.

Laura Garwin

Control of receptor appetite

John Whicher

In the past ten years a great deal has been learned about the complex family of cellular regulatory proteins known as cytokines. The family includes proteins variously described as lymphokines, monokines, interferons and interleukins, of which interleukin-1 (IL-1), first described in 1979, is the archetype. Despite a vast literature and ample evidence that they have far-reaching effects, there is still no firm understanding of the biological or pathological significance of the two forms of this cytokine, or of the consequences of its pharmacological manipulation. So it is of some interest that, on page 633 of this issue¹, Carter *et al.* describe further *in vivo* effects of a recombinant IL-1 receptor antagonist first cloned and expressed by Eisenberg *et al.* and reported in *Nature* earlier this year^{2,3}.

Two main forms of IL-1 (IL-1 α and IL-1 β) are known. They are not strictly homologous proteins (having only 25 per cent amino-acid sequence homology), but the two cloned and sequenced genes show much the same organization and the recombinant products bind to the same receptors (although they may do so by different sites). Recent reports suggest the existence of two different classes of IL-1 receptors of differing affinities and molecular weights — the higher-affinity one on T lymphocytes and fibroblasts, the other on bone marrow granulocytes, pre-B cells and macrophages^{4,5}. IL-1 β , the predominant form in human biological fluids, is produced primarily from macrophages. *In vitro* it induces the production of prostaglandins, proteases, adhesion molecules, acute-phase proteins, interleukin-2, interleukin-6 and ACTH, and activates B cells, monocytes and neutrophils. *In vivo* effects include fever, sleep, weight loss, steroid release and neutrophilia. It is this extraordinary variety of effects, many of which overlap those of other cytokines and hormones, that makes the role played by IL-1 in physiology and pathology so difficult to explain.

Inhibitory activities against IL-1 have been described from a variety of sources such as the urine⁶ and serum⁷ of febrile patients, malignant monocytic cell lines⁸ and monocytes infected with viruses⁹, stimulated by immune complexes¹⁰, or from patients with certain diseases¹¹. Many of these inhibitors have been identified only because of their ability to block thymocyte proliferation, and their mechanism of action has not been elucidated in molecular terms. Two groups have, however, pursued their own 'activities' to a molecular level and shown the inhibitors to be specific IL-1 receptor-binding proteins. Seckinger *et al.*¹², have shown that

the inhibitory activity they first found in the urine of patients with fever and monocytic leukaemia¹³ competes with IL-1 for cell-surface binding on T cells and that it also inhibits prostaglandin E₂ and collagenase production by fibroblasts and synovial cells.

More recently, following the earlier work of Arend *et al.*¹⁰, Hannum, Eisenberg and others from Synergen, working with Arend from the University of Colorado, have purified and cloned the gene for an inhibitor from human peripheral blood monocytes^{2,3}. The purified protein exists as a non-glycosylated and as two glycosylated forms of a novel peptide. The recombinant protein, designated IL-1ra, has a relative molecular mass of 17,115 (152 amino acids), and binds in a saturable manner to the receptor on EL4-6.1 cells with a similar affinity to IL-1 itself. Although showing only 26 per cent homology with IL-1 β and 19 per cent homology with IL-1 α , many of the identical residues between IL-1ra and IL-1 β occur in short stretches of amino acids towards the C-terminal end of the protein. Hydrophobicity plots in this area are remarkably similar for the two proteins, and when homologous residues are mapped onto the three-dimensional structure, they cluster around one vertex of the pyramidal molecule. In addition, IL-1ra messenger RNA is similar to that of IL-1 α and IL-1 β in having a large open reading frame encoding the protein, preceded by a short 5' untranslated region and followed by a long 3' untranslated region. All these pieces of evidence suggest a family relationship between IL-1ra and the IL-1s themselves, although IL-1ra is a true secreted protein with obvious signal sequences. Interestingly, its production by monocytes may be regulated separately from IL-1¹⁴. In terms of function, IL-1ra inhibits fibroblast prostaglandin E₂ release but does not bind to murine pre-B cells, meaning that it may bind selectively to one of the two forms of the IL-1 receptor. *In vivo* it inhibits IL-1-induced hypotension and acute-phase protein induction in rabbits¹⁵.

What then of the inhibitor described by Carter *et al.*? From the nucleotide sequence of the complementary DNA, this IL-1 receptor antagonist (IRAP) would seem to be identical to the IL-1ra of the Synergen group. The source of the original material was the human monocytic cell line U937, rather than human peripheral blood mononuclear cells. The contribution of Carter *et al.* lies not in confirming the previous findings in a slightly different way, but in demonstrating *in vivo* effects of the antagonist that

provide further evidence indicating selective binding to different forms of the IL-1 receptor. *In vitro*, the IRAP inhibits IL-1-induced adhesion of neutrophils and eosinophils to endothelial cells in a concentration-dependent fashion. It inhibits both IL-1 α and IL-1 β in the 1A5 and murine thymocyte IL-1 comitogenesis assays. *In vivo*, the IRAP suppresses corticosterone release when injected subcutaneously into the mouse hind paw along with IL-1 α or IL-1 β , or when administered intraperitoneally in mice receiving subcutaneous IL-1 α . Despite the bizarre experimental conditions, and the unusual time course of corticosterone release, this is an intriguing observation.

Perhaps of even greater interest, however, is the failure of the inhibitor to block the IL-1-induced neutrophilia in the same animals. The possibility that this may be due to binding to only one form of the IL-1 receptor is supported by the finding that IRAP competes with radiolabelled IL-1 α for binding to YT cells but not to mouse bone-marrow cells or 70Z/3 pre-B cells. The IL-1 receptors on YT cells, and mouse brain and pituitary cells have a similar K_d to the T-cell/fibroblast form.

The exciting features of the IL-1ra/IRAP are that it is a true antagonist of IL-1 α and IL-1 β , that it may be selective for certain cells and tissues, and that it is a third member of the IL-1 family of proteins. From existing evidence, it is now clear that the sensitivity of different cells to IL-1 might be conditioned by receptors of different affinity. The finding of an antagonist specific for one receptor class, produced along with IL-1 from monocytes, but regulated separately, adds further sophistication to the mechanisms controlling the actions of IL-1.

The significance of these findings lies in the possibility of using the IL-1ra/IRAP to unravel some of the puzzles that surround the action, *in vivo* and *in vitro*, of IL-1. We may then be in a position, as Carter *et al.* suggest, to consider whether the IRAP can be used as a therapeutic agent against inflammation. □

John Whicher is in the Department of Chemical Pathology and Immunology, University of Leeds, Old Medical School, Leeds LS2 9JT, UK.

1. Carter, D. B. *et al.* *Nature* **344**, 633–638 (1990).
2. Hannum, C. H. *et al.* *Nature* **343**, 336–340 (1990).
3. Eisenberg, S. P. *et al.* *Nature* **343**, 341–346 (1990).
4. Bomsztyk, K. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **86**, 8034–8038 (1989).
5. Chizzonite, R. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **86**, 8029–8033 (1989).
6. Liao, Z., Grimshaw, R. S. & Rosenzreich, D. L. *J. exp. Med.* **159**, 126–136 (1984).
7. Prieur, A.-M. *et al.* *Lancet* **ii**, 1240–1242 (1987).
8. Barak, V. *et al.* *Eur. J. Immun.* **16**, 1449–1452 (1986).
9. Rodgers, B. C. *et al.* *J. Virol.* **55**, 527–532 (1985).
10. Arend, W. P., Joslin, F. G. & Massoni, R. J. *J. Immun.* **134**, 3868–3875 (1985).
11. Westacott, C. I. *et al.* *Clin. Sci.* **75**, 561–567 (1988).
12. Seckinger, P. *et al.* *J. Immun.* **139**, 1546–1549 (1987).
13. Balavoine, J.-F. *et al.* *J. clin. Invest.* **78**, 1120 (1986).
14. Arend, W. P. *et al.* *Cytokine* **1**, 149 (1989).
15. Ohlsson, K. *et al.* *Cytokine* **1**, 131 (1989).

Ocean ridges spring surprises

Charles H. Langmuir

THE development of plate tectonics and early sparse observations of ocean ridges led to a simple and straightforward idea of how ridges worked. It was thought that ridges were linear spreading segments, periodically offset by transform faults. Slow-spreading ridges had small steady-state magma chambers and a central rift valley, whereas fast-spreading ridges had large, steady-state chambers and did not have a rift valley. A consequence of this simple view of ocean ridges is that a two-dimensional cross-section of a ridge was thought sufficient to describe its essential characteristics. New results from the slow-spreading Mid-Atlantic Ridge and the fast-spreading East Pacific Rise provide detailed observations that are inconsistent with aspects of these long-standing theoretical models of how slow- and fast-spreading ridges work. Kent and others,

on page 650 of this issue¹, raise the possibility that the magma body beneath the East Pacific Rise is in fact quite small. And Lin and others² (page 627), show that the most common offsets of the Mid-Atlantic Ridge are not transform faults, and that slow-spreading crust formation is a fully three-dimensional process.

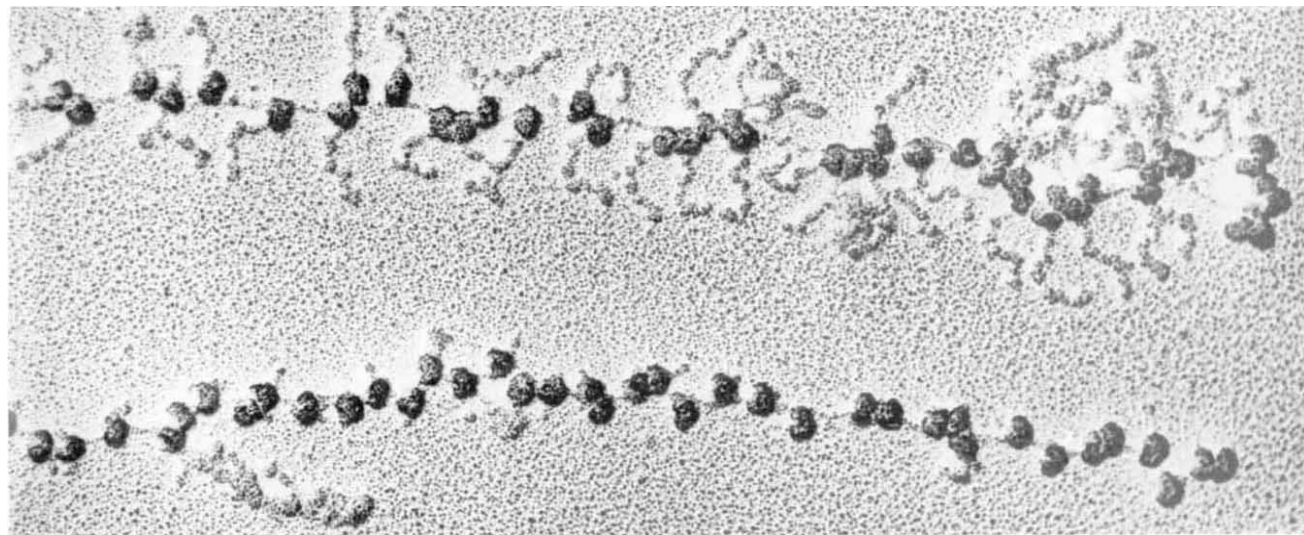
Estimates of the size of the East Pacific Rise magma chamber have decreased in recent years, based on results from studies of its bathymetry³, petrology⁴ and multichannel reflection seismology⁵. Kent and others constrain the size even further by a detailed analysis of multichannel seismic data from one of the currently most active portions of the East Pacific Rise near 9°30' N. Their analysis suggests that the magma body may be only a kilometre wide and a hundred metres thick, a size so small that it merits the

name 'melt lens' rather than magma chamber. Such a small body, supplied with magma from beneath the crust at multiple locations within a spreading cell⁴, would help to reconcile geophysical and petrological data from the East Pacific Rise^{4,5}. This melt lens is inconsistent with theoretical and ophiolite-based models of large, steady-state magma chambers at fast-spreading ridges. It calls for a new theoretical analysis of the heat budget at fast-spreading ridges. How can high-temperature magma be maintained in such a small magma body?

Lin *et al.*² and Sempere *et al.*⁶ report equally remarkable results from a recent survey of the Mid-Atlantic Ridge. This survey, which included detailed observations of bathymetry, gravity and magnetic anomalies over a substantial section of the ridge, is one of the first of its kind for a slow-spreading ridge.

The bathymetric results from between 24° N and 30° N (ref. 6) reveal a length of 800 kilometres of ridge where there are no transform offsets at all. The ridge contains

Seeing the cellular translators at work



E. V. Kiseleva

IF there remain any doubters that the genetic message is translated by ribosomes moving in order along messenger RNA, gradually extending their individual protein chains and thereby producing a gradient of polypeptide chain lengths, they will surely be converted by the evidence of this micrograph ($\times 140,000$), one of a number published by E.V. Kiseleva (*FEBS Letts* 257, 251–253; 1989). At bottom is the start of the polysome, at top the end, the rest of it looping out of shot.

This is not the first demonstration of its kind; similar pictures appeared in 1982 (Franke, C. *et al.* *EMBO J.* 1, 59–62; 1982). But the new pictures confirm that earlier work on the polysomes, and show our indebtedness to the lowly midge larva (*Chironomus*) for

furnishing suitable material — salivary gland protein secreting cells — for such demonstrations. The genes that make these messages have provided our best views of transcribing genes *in situ*, and also of the transit of the resulting messenger RNP particles through the pore complexes of the nuclear envelope.

The polysomes in Kiseleva's preparations have the added interest of being attached by their 5' ends to membranous structures, presumably derived from the endoplasmic reticulum. This new information supports theories of the 'solid-state' transfer of RNA molecules throughout their cellular history (Agutter, P. *Prog. mol. Subcell. Biol.* 10, 15–96; 1988). In this instance the mRNA is attached to the membrane, though the possibility remains that there is also some involvement of

the cytoskeleton, not preserved in the preparation.

The startling clarity of the pictures remains their greatest value. Ribosomes are frequently encountered in conventional transmission electron microscopy of thin sections, but that technique cannot reveal the detail of either the RNA itself, or the growing proteins attached to their ribosomes. The detail in Kiseleva's pictures enables us to see protein substructure; it is possible to count up to six beads towards the N-terminal end of the nascent polypeptides.

E.G. Jordan

E.G. Jordan is in the Biophysics Section, King's College London, 26–29 Drury Lane, London WC2B 5RL, UK.

many segments, but Lin *et al.* find that these segments often overlap one another, and are bounded by non-transform offsets. Similar offsets have been known in the Atlantic for some time⁷, but they were often thought to be poorly defined transform faults, rather than an indication of an entirely different style of ridge segmentation. Sempere *et al.*⁶ suggest that these non-transform discontinuities may be playing a similar role to the overlapping spreading centres discovered several years ago on the East Pacific Rise^{3,8}. The discontinuities divide the ridge into a series of separate magmatic systems.

Mantle Bouguer anomaly maps of the gravity data² from this region show a series of 'bullseyes' that correlate with the segmentation defined by the offsets. There are circular gravity lows near the shallow mid-points of most of the six spreading segments surveyed, and large positive gravity anomalies over the non-transform discontinuities. The magnitude and distribution of the mantle Bouguer anomalies are not consistent with the predictions of the thermal model of uniform mantle upwelling between fracture zones. Lin *et al.* suggest two possible explanations: either the crustal thickness varies by over 50 per cent from the centres to the edges of the segments; or the mantle beneath the centre of each segment upwells preferentially, leading to higher temperature and more melting, both of which would cause a decrease in the mantle density. These two possibilities are not mutually exclusive, in that both imply a focused supply of melt towards the centre of a spreading segment. The gravity and bathymetric data together show that the punctuation of the ridge by non-transform offsets is indicative of deep-seated along-axis periodicity in melt generation and supply.

Another exciting result of the Atlantic survey^{2,6} is the discovery of simple linear relationships between the length of a given segment, the total variation in the depth of the ridge, and the variation in the mantle Bouguer anomaly. Lin *et al.* suggest that this may be because longer spreading segments are associated with larger, more rapidly ascending mantle plumes.

A valuable aspect of this model of highly focused magma supply beneath the centres of slow-spreading ridge segments is that it provides precise predictions which can be tested. The most obvious will be determinations of crustal thickness variations both for a single segment, and for segments of different length. Certainly, the results call for a closer examination of the multichannel seismic reflection data on older crust.

There are testable predictions for the chemical composition of oceanic crust as well. Recent work on mid-ocean-ridge basalts has further raised the possibility

of constraining the extent and pressure of melting by using major-element chemistry⁹. Preferential upwelling of hotter mantle beneath the centres of ridge segments should lead to a greater extent of melting at lower pressures from the centres as compared to the edges of the segments. This should cause basalts from the centres of segments to have relatively low concentrations of Na₂O and FeO for a given MgO content. There are few data yet available to test this possibility. Batiza *et al.*¹⁰ showed that one spreading segment from the South Atlantic exhibits along-axis chemical systematics, but the results are more complicated than a simple central-injection model would suggest. In any case, future models will have to accommodate the data on gravity and crustal thickness with the spatial systematics of the chemical composition of the crust. The French-American Ridge

SOLAR PHYSICS

Sounding a small heliosphere

W.S. Kurth

How big is the Solar System? Pluto's orbit extends out to 50 times as far as Earth's, that is, 50 astronomical units (AU). But is there a planet X at a greater distance? Indeed, the hypothesized Oort cloud, suggested to be the source of comets, might exist at distances of 10⁵ AU (though this would be virtually unconnected with the Solar System). The solar wind, bearing the ions and electrons radiated from the Sun, extends out to the heliopause, the boundary formed where the wind runs into the interstellar medium (see figure). On page 640 of this issue¹, Czechowski and Grzedzielski suggest that the behaviour of radio waves inside the heliopause can be used to determine its extent. The size they estimate, 60–100 AU, is small compared to many estimates of the size of the heliosphere. The distance to the 'termination shock', where the solar wind becomes subsonic, could be as little as 50 AU, or

Atlantic project will include a more intensive study of this region of the Mid-Atlantic Ridge and should enable these questions to be addressed in depth during 1991. □

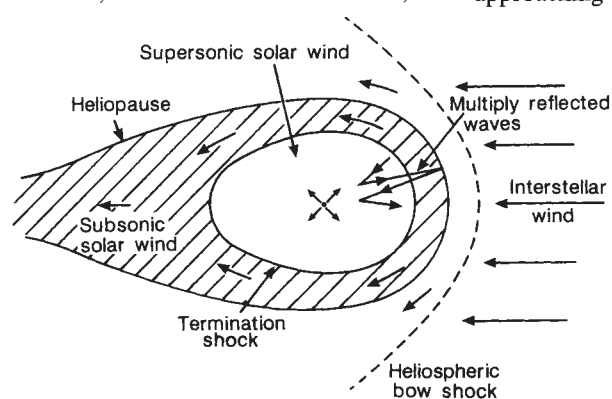
Charles H. Langmuir is at the Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York 10964, USA.

1. Kent, G.M., Harding, A.J. & Orcutt, J.A. *Nature* **344**, 650–653 (1990).
2. Lin, J., Purdy, G.M., Schouten, H., Sempere, J.-C. & Zervas, C. *Nature* **344**, 627–632 (1990).
3. Macdonald, K.C., Sempere, J.-C. & Fox, P.J. *J. geophys. Res.* **89**, 6049–6069 (1984).
4. Langmuir, C., Bender, J. & Batiza, R. *Nature* **322**, 422–429 (1986).
5. Detrick, R.S. *et al.* *Nature* **326**, 35–41 (1987).
6. Sempere, J.-C., Purdy, G.M. & Schouten, H. *Nature* **344**, 427–431 (1990).
7. Ramberg, I.B., Gray, D.F. & Reynolds, R.G.H. *Geol. Soc. Am. Bull.* **88**, 609–620 (1977).
8. Lonsdale, P. *J. geophys. Res.* **88**, 9393–9406 (1983).
9. Klein, E.M. & Langmuir, C.H. *J. geophys. Res.* **94**, 4241–4252 (1989).
10. Batiza, R., Melson, W.G. & O'Hearn, T. *Nature* **335**, 428–431 (1988).

less. This result is of great interest as Voyager 1 and Pioneer 10 are now at about 40 and 48 AU respectively.

The key to the new distance determination is an upward drift at a rate of about 1 kilohertz (kHz) per year in the frequency of 3-kHz interplanetary radio emissions observed by the Voyager plasma wave receivers^{2,3}. Czechowski and Grzedzielski assume that the radio frequencies are sufficiently low for the emissions to be trapped between the high-density inner heliosphere and the relatively high-density interstellar wind. The waves repeatedly reflect off the heliopause and high-density structures expanding away from the Sun. Since both reflection surfaces, particularly the expanding density structures, are in motion, the radio waves suffer Fermi acceleration, that is, their frequency increases or decreases depending upon whether the reflection surface is moving towards or away from the approaching radio wave at reflection.

Calculating the exact outcome of such Fermi accelerations and the intervening propagation is a complex analytical problem. The result of this calculation, as well as intuition, leads to the conclusion that reflections off density structures moving outwards from the Sun with the speed of the solar wind, typically 400 km s⁻¹, result in a net increase in frequency; reflections off the heliopause are stochastic and lead to no net frequency



Schematic showing the interaction of the solar wind, carrying particles radiated from the Sun (central dot), with the interstellar medium.

drift. Because the velocity of these expanding density structures is known, on average, very well (by numerous *in situ* observations by Voyager, Pioneer and other spacecraft), the primary unknown determinant of the frequency drift rate is the size of the cavity, which determines how often the reflections occur. Thus a very slow change in frequency would indicate a large cavity (few reflections), and a more rapid change, as is observed, indicates a smaller cavity in which there are many reflections per unit time.

This particular result is noteworthy because of its independence of the source of the radio emissions. Earlier estimates of the distance to the termination shock provided by these radio emissions^{2,4} were based on a particular generation mechanism and on the postulation that the radio waves were generated at the termination shock. The present work requires only that the waves are generated in the cavity and that they are at a frequency lower than the plasma frequency in the surrounding interstellar wind (that is, they are trapped in the cavity). There has been little recent progress in verifying the source of the low-frequency radio emissions. Of the several possible sources for these low-frequency waves that have been suggested, only two of them, pulsars and nearby stars, are from outside the Solar System, and neither possibility is thought likely.

Further, Czechowski and Grzedzielski offer an explanation for the frequency drift of the radio emission without requiring an *ad hoc* change in the source plasma or in the generation mechanism. Previous discussions of the frequency drift of the 3-kHz emission required changes in the source plasma frequency which, in turn, require a continuous change in the density of plasma at the source over a period of several months. Such a change is highly unlikely in or near the region of supersonic solar wind flows, such as near the termination shock, where density variations primarily follow the cycle of solar rotation with a period around 25 days. Should the source of the emissions be at or near the heliopause, such slowly varying conditions, caused by convecting density structures in the subsonic solar wind or by variations in the interstellar density, might occur. But there is no independent evidence for such variations.

The Fermi acceleration that causes frequency drift of radio emissions has already been successfully invoked to explain frequency variations of radio waves trapped in magnetospheric cavities⁵. In this case, the Fermi process smears out the spectrum of narrow-band radio emissions generated by upper hybrid resonance bands to form a quasi-continuous spectrum of the trapped continuum radiation in the Earth's magneto-

sphere. The movement of the Earth's magnetopause is stochastic, so there is no monotonic change in frequency. That a similar process takes place for radio waves trapped in the heliospheric solar cavity is no surprise. In the case of the solar wind, however, reflections off the continuously expanding density structures result in a net increase in frequency with each successive reflection.

The suggestion by Czechowski and Grzedzielski¹ — that a Fermi acceleration process explains the frequency drift of the 3-kHz interplanetary radio emissions — provides a new and independent measure of the distance to the heliopause. The

resulting distance of 100 AU or less means that the Voyager and Pioneer spacecraft could be returning the first observations of the subsonic solar wind in just a few years. □

W.S. Kurth is in the Department of Physics and Astronomy, University of Iowa, Iowa City, Iowa 52242, USA.

1. Czechowski, A. & Grzedzielski, S. *Nature* **344**, 640–641 (1990).
2. Kurth, W.S., Gurnett, D.A., Scarf, F.L. & Poynter, R.L. *Nature* **312**, 27–31 (1984).
3. Kurth, W.S., Gurnett, D.A., Scarf, F.L. & Poynter, R.L. *Geophys. Res. Lett.* **14**, 49–52 (1987).
4. Suess, S.T. & Dessler, A.J. *Nature* **317**, 702–703 (1985).
5. Barbosa, D.D. *Astrophys. J.* **243**, 1076–1087 (1981).

MOLECULAR PALAEOBOTANY

Turning over an old leaf

Karl J. Niklas

THE application of new techniques is continually pushing back the age of DNA that can be extracted and sequenced from samples of fossil tissue. But even the most optimistic estimates of the longevity of this molecule would not have predicted that fragments of substantial length would survive after tens of millions of years at the bottom of an ancient lake. Nonetheless, as reported on page 656 of this issue¹, Golenberg *et al.* have extracted and amplified a segment of DNA from fossil *Magnolia* leaves dating from 17–20 million years ago.

The DNA fragment was isolated by means of selective double-stranded DNA amplification from the chloroplast gene,

species — a research area that is at present characterized by too much speculation chasing too few data^{2,3}.

The exceptional preservation of the *Magnolia* leaves is attributed to the low oxygen content and cold temperatures of the water in which they were deposited. The leaves were recovered from a relatively thick sequence of lake-bottom sediments representing an anoxic depositional environment in the Clarkia fossil beds of northern Idaho⁴. The geochemistry and ultrastructure of numerous leaf fragments isolated from the beds indicate that leaves fell into the lake and remained unoxidized and water-saturated to the present day^{5,6}. When rocks containing



Still green — fossilized *Magnolia latahensis* leaf (courtesy E. M. Golenberg).

rbcL, which encodes the large subunit of ribulose 1,5 biphosphate carboxylase/oxygenase ('rubisco'). Perhaps even more remarkable than the sheer age of the fragment is that the authors were able to compare it cladistically with sequences of DNA fragments containing the *rbcL* gene from extant species. As well as demonstrating the possibility of very precise molecular palaeontology, this work opens the door to the rigorous testing and calibration of mutation rates among related

these fossils are cleaved open, the freshly exposed leaf tissues are often bright green or 'deep autumnal' in colour, though they rapidly darken and curl away from the substrate as they oxidize and dry out.

The segment of the chloroplast gene preserved in the fossils codes for part of 'rubisco', the commonest protein (from one-half to three-quarters of the soluble protein fraction) found in the chloroplast. Because plants contribute over 90 per cent of the Earth's biomass, the protein is

probably the most abundant in the world. Significantly, Golenberg *et al.* are not merely content to demonstrate that DNA can survive for an extended period of geological time, but also show that differences in the sequence of the *rbcL* gene can be used to estimate phylogenetic relationships among plants. Based on the morphology of its leaves and that of gynoecia (the reproductive organs bearing ovules), the fossil *Magnolia* was identified as *M. latahensis* (Berry) Brown. The fossil is similar to three living species of *Magnolia* (*M. grandiflora*, *M. macrophylla* and *M. virginiana*) from eastern North America. Golenberg *et al.* compared the *rbcL* sequence of the fossil with those of 18 extant species and found that the fossil DNA sequence never grouped outside that of the other Magnoliidae species examined. The fact that the fossil sequence clustered with, but differed from, those of other *Magnolia* species provides confirmation that it comes from a distinct but related species. In addition, these comparisons substantiate the view that the fossil DNA fragments are not the result of chemical contamination from living plant material.

Scientists have always striven for in-

dependent lines of evidence with which to test hypotheses, none more than palaeontologists whose discipline has been sometimes characterized as 'non-experimental' and 'resistant to falsification'. The new-found ability to recover chemical compounds from extremely ancient biological remains, and to use chemical differences between fossil plants and animals to explore taxonomic relationships and rates of evolution, provides information independent of morphological and anatomical data. The latest demonstration of the power of that ability by Golenberg and colleagues gives palaeontologists and molecular biologists even more cause to talk to each other. □

Karl J. Niklas is in the Section of Plant Biology, Cornell University, Ithaca, New York 14853, USA.

1. Golenberg, E.M. *et al.* *Nature* **344**, 656–658 (1990).
2. Li, W.-H., Guoy, M. & Wolfe, K.H. *Nature* **342**, 131–132 (1989).
3. Crane, P.R., Doyle, J.A. & Friis, E.M. *Nature* **342**, 131 (1989).
4. Smiley, C.J. (ed.) *Late Cenozoic History of the Pacific Northwest* (AAAS, San Francisco, 1985).
5. Giannasi, D.E. & Niklas, K.J. *Science* **197**, 765–767 (1977).
6. Niklas, K.J., Brown, R.M. Jr. & Santos, R. in *Late Cenozoic History of the Pacific Northwest* (ed. Smiley, C.J.) 143–159 (AAAS, San Francisco, 1985).

quantum-mechanical calculations^{1,2} is remarkable. Furthermore, the comparison shows that the predictions obtained by applying classical mechanics to the reaction slightly overestimate the amount of rotation of the DH products.

Quantum-mechanical calculations on the dynamics of chemical reactions involve two stages. First, the potential energy surface has to be computed. This is the electronic energy of the system expressed as a function of the distances between the atoms involved. Significant progress in *ab initio* quantum chemistry has made possible the calculation of a highly accurate potential energy surface for the $H+H_2$ reaction¹⁰. The second stage in the theory involves solving the Schrödinger equation for the three atoms moving in this potential field. It is only recently that general methods for performing such molecular-scattering calculations have been developed.

Back in 1976, a landmark paper was published by George Schatz and Aron Kuppermann¹¹. In calculations done at the California Institute of Technology, they obtained converged results for the $H+H_2 \rightarrow H_2+H$ reaction at low collision energies with an approximate potential energy surface. Their calculation suggested that 70 per cent of the reaction at room temperature occurs because of quantum-mechanical tunnelling through the potential energy barrier, which should otherwise keep insufficiently energetic reagents apart. The authors used a set of natural collision coordinates to describe this reaction that take advantage of the special symmetry of the problem. But, despite intensive efforts, it has not proved straightforward to extend this method even to the $D+H_2$ reaction and it has been necessary to formulate alternative theories. For example, a set of polar coordinates has been developed that avoids the difficulties of the natural collision coordinates and has enabled calculations to be performed on several reactions³.

Furthermore, in a completely different approach, theories have been developed independently by Bill Miller and John Zhang at Berkeley¹, and in a collaboration between the groups of Don Truhlar at Minnesota and Don Kouri² in Houston. These techniques have provided the most extensive results to date including those on the $D+H_2$ reaction that compare so well with those obtained in experiments of the Zare group. These theories borrow ideas used in the molecular orbital theory of quantum chemistry, in that the solutions are obtained as basis set expansions in the vibrational and rotational quantum states of both the reactant and product molecules. However, it is only through the use of large supercomputers, such as the CRAY-2, that calculations with these methods have been possible.

Alternative experimental methods also

Solutions to simplest systems

David C. Clary

CHEMICAL reactions should, in principle, be exactly describable in terms of quantum mechanics. It is only now, however, that highly accurate calculations have been made for the simplest of chemical reactions, $H+H_2 \rightarrow H_2+H$ and its isotopic equivalent $D+H_2 \rightarrow DH+H$, where D is a deuterium atom^{1–6}. These predictions need to be checked by comparison with measurements, but detailed experiments on such simple reactions are as hard to perform as the calculations, owing to the difficulties of detecting H_2 molecules and problems in producing a beam of atoms with a precise energy. These experimental difficulties have now been overcome through the application of sophisticated laser techniques^{7,8}. Indeed, the new experimental paper in *Chemical Physics Letters* from the group of Dick Zare at Stanford University⁹ highlights the outstanding agreement that can now be achieved between experimental results and theoretical predictions in this field.

Elucidating the details of individual steps in chemical reactions has long been a leading topic in chemistry. As experimental and analytical techniques have improved, it has become possible to inspect reactive collisions between molecules with remarkable detail (see Ian W. M. Smith's recent News and Views article⁹ on 'femtosecond' chemistry).

It is now feasible to study reactions in which both the reactant and product molecules are selected in their individual vibrational and rotational quantum states.

The new Stanford experiment on the $D+H_2$ reaction uses many of the methods familiar in this field (see figure). A laser beam is used to dissociate DBr molecules to produce a beam of fast deuterium atoms that are reacted with hydrogen molecules. The product DH molecules

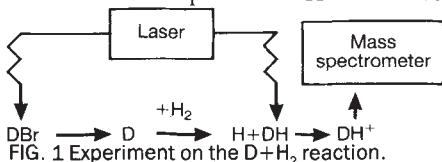


FIG. 1 Experiment on the $D+H_2$ reaction.

produced are detected by being ionized with the same laser beam. Measuring the time the ionized molecules take to pass through a mass spectrometer, so revealing their kinetic energy, enables Zare and colleagues to deduce the probability of producing the product DH molecules in various vibrational-rotational (v, j) quantum states. The most likely DH rotational state produced in the first vibrational level is $j = 4$, although as many as nine such rotational states can be populated. The agreement between the probability distribution of the DH product obtained in this measurement and that obtained from

exist and one used by Jim Valentini and colleagues at the University of California, Irvine, has provided particularly interesting results'. This 'CARS' ('coherent anti-Stokes Raman spectroscopy') method involves simultaneously using two laser beams, one of fixed frequency and one tunable, to detect the product molecules of the chemical reactions. Early applications were to the $\text{H} + \text{D}_2 \rightarrow \text{HD} + \text{D}$ reaction, for which accurate quantum results were not available for comparison. More recent experiments have been done on the simpler $\text{H} + \text{H}_2$ reaction and surprisingly suggest that the reaction cross-sections do not increase smoothly with the collision energy. This has been attributed to 'scattering resonances' associated with the temporary trapping of the reaction in the H_3 transition state at particular collision energies. Such resonances were found previously in calculations in which the geometry of the $\text{H} + \text{H}_2$ reaction was restricted to one dimension. But recent calculations suggest that the three-dimensional aspects of the reaction average out the resonance effects and the cross-section should increase smoothly with collision energy^{4,5}. Further experiments are required using different techniques to examine this discrepancy between theory and experiment.

What are the possibilities for predicting highly accurate results for reactions more complicated than $\text{H} + \text{H}_2$? The scattering theories have been extended recently to the reactions of H_2 with O, F and Br atoms, but a significant advance in computer power will be needed before the potential energy surfaces can be calculated accurately for reactions such as these. What the calculations and experiments on the $\text{H} + \text{H}_2$ reaction have done is to provide benchmark results that will enable the accuracy of new theories and experiments to be checked before they are applied to more complicated systems. They also show that quantum mechanics will be needed for an accurate description of the details of many chemical reactions. □

David C. Clary is in the Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK.

1. Zhang, J.Z.H. & Miller, W.H. *J. chem. Phys.* **91**, 1528–1547 (1989).
2. Zhao, M., Truhlar, D.G., Schwenke, D.W. & Kouri, D.J. *J. phys. Chem.* (in the press).
3. Pack, R.T. & Parker, G.A. *J. chem. Phys.* **90**, 3511–3519 (1989).
4. Zhang, J.Z.H. & Miller, W.H. *Chem. Phys. Lett.* **153**, 465–470 (1988).
5. Manolopoulos, D.E. & Wyatt, R.E. *Chem. Phys. Lett.* **159**, 123–129 (1989).
6. Park, T.J. & Light, J.C. *J. chem. Phys.* **91**, 974–988 (1989).
7. Nieh, J.-C. & Valentini, J.J. *J. chem. Phys.* **92**, 1083–1097 (1990).
8. Kliner, D.A.V., Rinnen, K.-D. & Zare, R.N. *Chem. Phys. Lett.* **166**, 107–111 (1990).
9. Smith, I.W.M. *Nature* **343**, 691–692 (1990).
10. Siegbahn, P. & Liu, B. *J. chem. Phys.* **68**, 2457–2465 (1978).
11. Schatz, G.C. & Kuppermann, A. *J. chem. Phys.* **65**, 4668–4692 (1976).

The talk of the Americas

Jared M. Diamond

THE largest expansion of human *Lebensraum* in the past half-million years was the colonization of the New World by predecessors of modern American Indians ('native Americans'). If we knew when, whence and in how many waves those colonists had arrived, we would have the key to several questions in human genetics, anthropology and linguistics. A drastic and heavily criticized reclassification of native American languages

University of Texas Press, 1979).

Enter Greenberg (Stanford University, Stanford), whose reclassification of African languages was also highly controversial but is now praised and accepted. After similarly revising the morass of New Guinea languages, Greenberg turned his attention in the 1950s to the Americas. In each case he employed a broad-brush method termed multilateral (or mass) comparison, in which whole sets of pos-

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Past cultural glory — a native American in traditional head-dress.

by Joseph Greenberg (*Language in the Americas*, Stanford University Press, 1987) bears upon all of these questions. At a conference in Colorado — an appropriate venue for an academic shootout in the Old West — scholars from diverse fields assembled to assess Greenberg's hypothesis*.

To grasp the difficulty of the problem, reflect that about a thousand native American languages have been described, and as many more may have disappeared between the arrivals of European explorers and of linguists. Sister languages diverge with time so that relationships become difficult to detect after about 6,000 years, yet America was colonized at least 11,000 years ago. Most scholars recognize native American languages as falling into about 150 distinct families. They are sympathetic to the likelihood of relationships among those families, but they conclude that techniques for detecting them after such a long time are inadequate (L. Campbell and M. Mithun *The Languages of Native America*,

sibly related languages, rather than just selected pairs, are compared with respect to vocabulary and grammar. Greenberg's reasoning is that each sister language of a family may lose different inherited features, with the result that family properties and membership can be deduced only from study of the whole set. Protein chemists who have studied evolutionary relationships among retroviruses or acyl-amino-acid transfer-RNA synthetases will immediately appreciate this reasoning.

Greenberg concluded that all native American languages belong to one of three stocks (see map, over). Two of these are small North American stocks already recognized by most linguists: Eskimo-Aleut and Na-Dene, consisting of about nine and 34 languages respectively. What is controversial is Greenberg's conclusion that all other native languages in North and South America belong to one gigantic stock which he terms Amerind.

Most linguists reject not only Greenberg's conclusions but also his methods. Instead, they consider recognition of sound correspondences between lan-

**Language and Prehistory in the Americas*, Boulder, Colorado, 22–25 March, 1990.

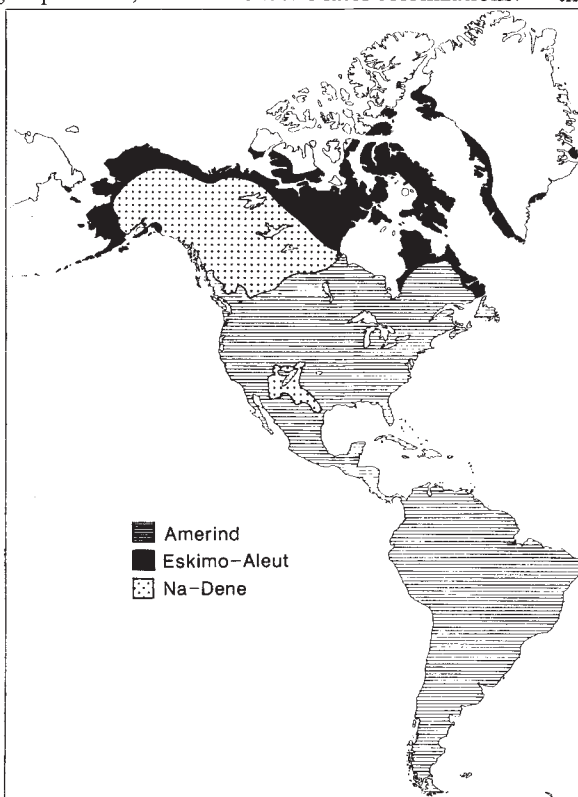
guages (for example the English 'th' and the German 'd') as a prerequisite for identifying relationships. Without such recognition, one might not recognize that 'star' (English) and 'étoile' (French) are derived from the same proto-Indo-European root. L. Campbell (Louisiana State University, Baton Rouge) objected that Greenberg's methods would have classified Finnish as Amerind if some native American group had spoken Finnish, while S. Starostin (USSR Academy of Sciences, Moscow) noted a large number of errors resulting from the neglect of sound correspondences in some of Greenberg's comparisons. Greenberg's response was that one cannot search for sound correspondences until one has a hypothesis about which languages to compare, and that recognition of now-accepted stocks such as Indo-European preceded rather than followed the study of sound correspondences. Nevertheless, sound correspondences will surely be the route pursued by most other linguists for testing or refining Greenberg's conclusions.

The issues dividing Greenberg and his critics flared up most often in an argument over pronouns used in the native American languages. Greenberg noted first- and second-person pronouns based on 'n-' and 'm-' respectively in all branches of his Amerind family. In one type of attack this claim was dismissed as mere 'mama/papa stuff' and not indicative of language relations. That is, 'm' and 'n' are particularly stable consonants, among the first to be pronounced by infants (possibly as a result of infant muscular activity and suckling noises), and hence are likely to lead not only to the almost worldwide term 'mama' but also to preference for 'm-' and 'n-' in pronouns (J. Nichols, University of California, Berkeley, and L. Campbell). Not explained by this point is why such 'mama/papa stuff' would affect pronouns especially in the Americas. So a second criticism was that 'n-' and 'm-' pronouns are not peculiarly American. Campbell reported ten sets of examples from non-American languages from half-an-hour's browsing in the library, though Greenberg described such pronouns as disproportionately common in the Americas, and M. Dryer (State University of New York, Buffalo) computed an incidence of 17 per cent in the Americas, 7 per cent elsewhere.

Astonishingly to non-linguists at the conference, none of the disputants had calculated whether there is a statistically significant difference in 'n-' and 'm-' pronoun frequencies between native American and other languages. In fact, use of even the most elementary statistics was

conspicuously absent at the conference, as it is from the tradition of historical linguistics, which must contribute to linguists' difficulty in resolving questions about whether languages A and B or A and C are more closely related.

If Greenberg's hypothesis is correct, it implies that the vast majority of native Americans derived from a single colonization, the rest from two later colonizations.



Distribution of Greenberg's main American language stocks.

This suggestion has stimulated great interest among geneticists, anatomists and archaeologists. Genetic evidence provided by L.L. Cavalli-Sforza *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **85**, 6002-6006; 1988), as well as evidence from tooth morphology (C. Turner, Arizona State University, Tempe), appears to be strikingly concordant with Greenberg's tripartite view. If the first colonists of America were the so-called Clovis hunters of 11,000 years ago, as some archaeologists believe, proto-Amerind might have been the Clovis language. But there is increasing evidence for pre-Clovis occupation of the Americas (D. Stanford, Smithsonian Institution; R. Gruhn, University of Alberta, Edmonton; T. Dillehay, University of Kentucky, Lexington). If Greenberg is correct about the unity of the Amerind stock, and if archaeologists can date the first human arrival, linguists will then have a unique opportunity to calculate the rate of language evolution over much longer times than those of the oft-cited diversifications of the Bantu and Polynesian languages.

Other predictions follow from Green-

berg's tentative conclusion that the Amerind stock is comprised of 11 subgroups of languages. Many subgroups have strikingly disjunct distributions, implying long-distance movements followed by massive extinction and replacement. For example, Greenberg construes the Paezan subgroup of South America as having a remote outlier in Florida, while the Gulf-Penutian group of the southeastern United States has an outlier in California. These controversial proposals will receive especial scrutiny from linguists, but they also beg for archaeological confirmation of the movements that could have produced them.

As controversial as Greenberg's tripartite theory, and his subgroupings within Amerind, are his attempts to connect his three native American language stocks with stocks of northeast Asia, from which the Americas must have been colonized. Given the difficulties of recognizing stocks that broke up at least 12,000 years ago, it is even more daring to try to identify putative 'macrostocks' that broke up earlier. Most linguists consider that such identifications are not feasible, a view that Greenberg dismisses as belief in the "immaculate conception of Indo-European". Greenberg, Starostin and others are pursuing this work and have thereby won themselves the equally dismissive label of 'supergroupers'. Greenberg's present view is that Eskimo-Aleut is a member of, and Amerind more distantly related to,

a widespread Eurasiatic macrostock that he sees as including Indo-European, Uralic, Altaic, Japanese and four other stocks. Na-Dene may be related to Sino-Tibetan. Thus the first arrivals, the Amerinds, occupied the whole hemisphere; the second arrivals, the Na-Denes, are still largely confined to northwest North America; and the most recent arrivals, the Eskimo-Aleuts, still live only at the latitudes of their far-northern port of entry into Alaska from Siberia.

Whether Greenberg's supporters or his much more numerous critics prove correct, the debate marks a new era in linguistics. With the present explosion of molecular genetic studies of human evolution, historical linguistics is moving back to centre stage. □

Jared M. Diamond is in the Department of Physiology, University of California Medical School, Los Angeles, California 90024, USA.

■ Those interested in the study of native American languages should turn to "Then and Now" (back page) for an intriguing slice of the history of their subject.

Face to face with proteins

Stephen Mann

THE chemical and electronic properties of surfaces and interfaces are subtly influenced by the nature of adsorbed species, so controlling the way that molecules interact with a solid support is crucial if reproducible interfaces are to be fabricated for catalytic, electrical and sensory devices. This problem is becoming increasingly severe as micrometre-scale devices are replaced by systems of nanometre scale. In general, crystalline substrates can act as templates for the site-directed adsorption of small molecules and ions, but there are difficulties in achieving this level of specificity with species of higher molecular weight such as proteins and polysaccharides. A report by Douglas *et al.*¹ now shows how macromolecules can be assembled into periodic arrays on the surface of a thin (1.2-nm-thick) tantalum/tungsten film. The solution was to design a polycrystalline metal-oxide surface containing a regular lattice of holes 14 nm in cross-section. The protein molecules (12 nm in diameter) then slot into the surface indentations by preferential electrostatic bonding.

Although the idea is simple, engineering a metal surface on the nanometre scale to provide a molecular screen is a complicated business. First, a suitable template has to be found. For this, Douglas *et al.* exploited the natural ability of certain bacteria to produce thin protein films of mesh-like crystal structure. They used the crystalline glycoprotein (relative molecular mass 140–170 K) present in the surface layers of the cell wall of the thermophilic bacterium *Sulfolobus acidocaldarius*. This protein has a structure based on a triangular lattice that contains large (14 nm) channels of sixfold symmetry repeated at distances of 22 nm (ref. 2). Monolayer fragments of the protein were mounted onto amorphous carbon substrates and then coated in a fine-grained film of 3-nm tantalum/tungsten particles by evaporation. Regularly spaced holes in this alloy film were then introduced by bombarding the surface with argon atoms (argon-ion milling), which selectively removed metal atoms overlying the original holes of the protein lattice³.

Assembling macromolecules into this 'nanofabricated screen' is not just a matter of fitting the right size molecule into the right size hole. Proteins will adsorb on the metal surface as well as in the holes, and the resulting surface structure becomes ill-defined. To overcome this obstacle, Douglas *et al.* made the alloy surface negatively charged by oxidation of the metal in air over a period of several months. This 'primed' surface was exposed to an aqueous solution of the anionic

protein, ferritin. Protein molecules were electrostatically repelled from the oxide surface except at sites occupied by the holes of the screen. The resulting structure is a unique composite of biomolecules and metal-oxide particles organized at the nanometre level.

There are some problems with the technique. First, the dimensions of the metal lattice tend to fluctuate because of the presence of trace amounts of organic contamination and redeposition of metal atoms during the argon-ion milling process. Second, the number of adsorption sites filled by the macromolecules is low, often only 15 per cent. Douglas *et al.* suggest an intriguing strategy to improve on the efficiency. The approach is to increase the activity of the adsorption sites by adding functional groups to the carbon substrate located at the bottom of the holes so that these regions become selective in terms of molecular recognition. Possibilities include the attachment of organic residues through coupling agents (silanes for example) and chemical conjugation by adsorbed antibodies. Alternatively, it may be possible to isolate inorganic clusters in the holes to give an organized heterometallic surface. Islands of gold, for instance, could then serve as periodic sites for binding biological molecules.

The approach adopted by Douglas *et al.* highlights the possibility of designing interfaces with organized biological and

solid-state components. It is unclear what immediate applications could arise from their work, but it is undoubtedly of fundamental importance in the study of controlled interfacial phenomena.

It is interesting to speculate whether analogues of these nanoheterostructures exist in biological systems. For example, model systems of biological membranes such as phospholipid vesicles can be metallized by metal⁴ and semiconductor particles⁵ of nanometre size, and compressed surfactant monolayers can act as templates for orientated crystal nucleation⁶. Could similar inorganic nanoclusters exist on the membrane surfaces of cells and organelles? It would be difficult to identify these components because they are so small (and because of the enthusiasm of biochemists for purified biomolecules). Yet such structures could be involved in redox, photochemical and catalytic processes. Moreover, the question arises whether associations of mineral clusters and self-assembled macromolecules served as energy-capture and transformation devices in the early stages of life. □

Stephen Mann is in the School of Chemistry, University of Bath, Bath BA2 7AY, UK.

1. Douglas, K., Clark, N.A. & Rothschild, K.J. *Appl. Phys. Lett.* **56**, 692–694 (1990).
2. Taylor, K.A., Deatherage, J.F. & Amos, L.A. *Nature* **299**, 840–842 (1982).
3. Douglas, K., Clark, N.A. & Rothschild, K.J. *Appl. Phys. Lett.* **48**, 676–678 (1986).
4. Ferrar, W.T., O'Brien, D.F., Warhowsky, A. & Voycheck, C.L. *J. Am. chem. Soc.* **110**, 288–289 (1988).
5. Youn, H.-C., Baral, S., & Fendler, J.H. *J. phys. Chem.* **92**, 6320–6327 (1988).
6. Mann, S., Heywood, B. R., Rajam, S. & Birchall, J. D. *Nature* **334**, 692–695 (1988).

HUMAN EVOLUTION

The Y of human relationships

John Maynard Smith

WHEN, three years ago, Cann *et al.*¹ estimated that all human mitochondria are copies of the mitochondria in one woman living 200,000 years ago in Africa, it was perhaps inevitable that this should have been reported in the press as "Eve lived in Africa 200,000 years ago". Since then, I have been looking to the day when studies of the Y chromosome led to the conclusion that Adam lived in China at an earlier or later date. Two publications^{2,3} on the human Y, one of which appears on page 663 of this issue², raised my hopes, but it seems that, as yet, the Y chromosome is less informative than the mitochondrion.

I must first explain how data of this kind can be interpreted. Suppose we have a population of entities (Y chromosomes, mitochondria) that reproduce without recombination, then all the entities in the present generation must be descended, with or without mutation, from a single

entity in some past generation. If we assume that selection is weak, we can estimate how many generations ago that common ancestor lived. If family size is Poisson distributed — a reasonable assumption — then the expected time to a common ancestor is $2N$ generations, where N is the number of entities in a single generation: unfortunately, this estimate has a large standard error. If the variation in family size is greater than Poisson, the expected time to a common ancestor is shorter.

Cann *et al.* constructed a phylogenetic tree of the mitochondrial DNA from 147 humans, using 195 polymorphic restriction types. The average difference between unrelated humans was 0.57 per cent of nucleotide divergence. They estimated the rate of sequence divergence in mtDNA as 2–4 per cent for each million years: this estimate was based partly on data from other mammals, and partly on

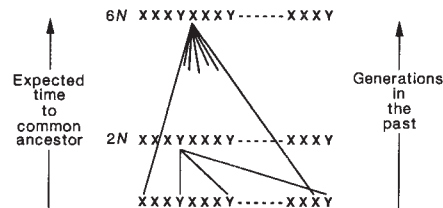
the amount of divergence that has occurred since humans entered New Guinea, Australia and the New World. Hence they estimated the time to a common ancestor as approximately 200,000 years, or 10,000 generations: note that this estimate does not depend on any assumptions about family size distribution, or absence of selection. But if we do assume no selection and a Poisson distribution, we can estimate that the human population contained, very approximately, 5,000 women. The number has of course fluctuated: the value of 5,000 should perhaps be taken as the geometric mean. Hence Eve, the woman who carried the ancestral mitochondrion, may have lived 200,000 years ago, but she had many female companions, and a like number of males, most of whom have contributed nuclear genes to the present population.

In principle, it should be possible to derive similar estimates from the Y chromosome, but this is proving difficult, because much of the DNA is highly repetitive, and the low copy number DNA is much more uniform than mtDNA, mainly because the mutation rate is higher in mitochondria. Ellis *et al.*² have tried to overcome this difficulty by sequencing 300 base pairs from the 'pseudoautosomal' region, adjacent to the boundary with the sex-specific region. This is the region where X and Y chromosomes have homologous sequences: although recombination does occur, it is rare, so that alleles now found only on the Y probably arose by mutation on the Y, and similarly for the X. Ellis *et al.* sequenced 57 Y and 60 X chromosomes, from ten human populations. They found seven variable sites: of these, four were polymorphic only on the X, one was polymorphic on both X and Y, and two were monomorphic for different bases on the X and Y. They conclude that the Y boundary region originates from an ancestor living more recently than does the X boundary region.

This conclusion is probably correct, although the numbers (five polymorphisms as opposed to one) are small. As the authors point out, this is to be expected on theoretical grounds (see figure). In a single generation, if there is a 1:1 sex ratio, there are three times as many X as Y chromosomes. Hence the expected time to a common ancestor is three times greater for the X, and the expected polymorphism is also greater. Their data on the distribution of haplotypes among X chromosomes suggest that this region of most human X chromosomes has descended from only two chromosomes with a long independent earlier history. This could prove to be of importance in elucidating patterns of migration.

In an independent study of the human Y chromosome (ref. 3), a low copy number region of the sex-specific Y was found to be polymorphic for five *TaqI* restriction

sites. The authors argue that, in a mammal, the presence of a *TaqI* site is likely to be primitive relative to its absence, because the CpG doublet is being eliminated in mammals (the *TaqI* endonuclease recognizes TCGA). If this is true, then the primitive allele can be recognized at each site. It is interesting that the chimpanzee has the primitive allele at four sites, and is polymorphic at the fifth. But this argument should be treated with caution. It is true that CpG is



Time to a common ancestor. The expected values, $2N$ and $6N$, have large standard errors. (X and Y are the female and male chromosomes; N is the number of males, and of females, in a single generation.)

rare in mammals because, due to methylation, it is readily lost by mutation. But if, as seems tenable, CpG has reached an equilibrium frequency, then loss and gain of such doublets will be equally common.

At present, the Y chromosome data are insufficient to make possible estimates of population size, or of the date and place of a common ancestor. Perhaps the most useful information provided by entities that are inherited without recombination concerns the history of past migrations: recombination destroys such information. The Y chromosome data agree with those on mitochondria in showing that most variation is present within racial groups. From this it would seem that most of the present variation was already present in an ancestral human population, before descendants of that population migrated world-wide. Cann *et al.*¹ suggest that Africa was the place of origin, essentially because, in their mitochondrial phylogeny, one main branch consists of Africans only. The Y chromosome data are compatible with an African origin, but as yet can provide only weak confirmation. In any case, if a value of 200,000 years (or even a value twice as great) is accepted for the ancestral mitochondrion, this would rule out the possibility that existing human populations are descended, in large part, from *Homo erectus* populations previously living in the same regions. □

John Maynard Smith is in the School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, UK.

1. Cann, R.L., Stoneking, M. & Wilson, A.C. *Nature* **325**, 31–36 (1987).
2. Ellis, N. *et al.* *Nature* **344**, 663–665 (1990).
3. Lucotte, G. in *The Human Revolution* (eds Mellors, P. & Stringer, C.) 39–46 (Edinburgh University Press, 1989).

A perfect spy

ELECTRONIC surveillance is normally a hostile technology, used with ill intent against others. Daedalus, however, is developing a self-surveillance device as a consumer product. DREADCO's 'Little Brother' looks like a tiny tape recorder. But it is worn next to the skin, and functions more like an aircraft flight recorder, or the tachograph on a lorry.

Little Brother records many tracks of data simultaneously, such as time, location (deduced by the relative intensity of the local broadcasting transmitters) and instantaneous acceleration (activities like walking, running, driving, and so on, have very characteristic accelerational signatures). Further tracks record ambient sound and the wearer's own speech, and physiological data like his temperature and pulse. Modern digital coding can pack at least a month's worth of such data on one tape; so Little Brother, which continuously overwrites the same tape with new data, always holds exact details of the last month of the wearer's life. Any event which turns out to have been significant can be saved for examination.

The main advantage will be legal. In the present litigious climate, anybody may suddenly need to prove any detail of his life in court. Little Brother will be an unimpeachable witness. A single tape track is easily falsified. Two tracks together are far trickier (which is why a sound-and-vision recording is hard to edit). The dozen or so tracks on Little Brother's tape will be utterly unfakeable. The wearer will be able to prove where he was at any time, how he got there, what he said and what was said to him, even (from the pulse-rate record) how furious he was.

Little Brother will have a salutary social effect. Crimes like confidence trickery and false accusation, together with shady deals and malicious gossip of all kinds, will simply vanish. Vexatious litigation will cease, and most honest disputes will be cleared up out of court by comparing the adversaries' Little Brothers. Policemen, sociologists and coroners will have a new source of evidence!

Once most people have a Little Brother, those who fail to carry one will automatically attract suspicion. Unfaithful husbands, secret gamblers and alcoholics, and tax-evading moonlighters will find life increasingly difficult. Ultimately, by making secrecy impossible, Little Brother may also make it unnecessary. Society will cease to set impossible standards for its members, and will tolerate their weaknesses and failings. But till that happy day, Daedalus is fitting his creation with an emergency-erase facility. A wearer suddenly caught in a truly impossible situation can scrub the whole tape instantly. His Little Brother will never testify against him. David Jones

Cuban K/T catastrophe

SIR—The possibility of a Caribbean location for the asteroid or comet impact that marked the end of the Cretaceous period was first raised by Pszczolkowski¹. Recently, Hildebrand and Boynton² have suggested that a half-metre-thick unit at the marine Cretaceous/Tertiary (K/T) boundary in Haiti, containing abundant altered microtektites and tektites, is an ejecta layer resulting from an impact in the Colombian basin.

This ejecta unit, originally described as a volcanogenic turbidite³, and later found to contain an iridium anomaly⁴, is almost 20 times thicker than similar non-marine units described from the western interior of North America. Our measurement of shocked quartz grain sizes had previously indicated that the site of the K/T impact must have been near the North American continent^{5,6}. The thickening of the impact ejecta layer southward among the North American sites⁷, together with the unusual thickness of the Haitian ejecta layer, enlarged our curiosity about a possible impact site in the Caribbean region.

The geological literature of the Greater Antilles reveals that Palmer⁸ described the uppermost member of the Cretaceous succession at the K/T boundary in Cuba as the "Big Boulder Bed". This remarkable unit contains near its base huge (up to 12-metre⁶) clasts and smaller boulders of limestone, sandstone and exotic igneous and metamorphic rock types, in a finer matrix. The exotic lithologies are mostly alien to the pre-Cenozoic geology of Cuba, as exposed today. Furthermore, of the three members of the Upper Maastrichtian (uppermost Cretaceous) described by Palmer⁹, only the Big Boulder Bed is found in every province of Cuba.

The average size of the boulders in this unit increases from north to south across the island, indicating a southerly source⁹. Beds containing this unit reach 350 m in thickness¹, and include serpentinite bodies several hundred metres in length⁸. A boulder bed 52 m thick has also been encountered at the top of the Cretaceous in offshore drilling on Pedro Bank, southwest of Jamaica¹⁰.

Underlying the Big Boulder Bed (revised and renamed in more recent publications¹¹) in Pinar del Rio and La Habana provinces is a more calcareous sandstone unit containing inverted cone-shaped "concretions" (ref. 9), with their bases lying parallel to the bedding planes. Palmer named this unit the "Cone Sandstone Member" and ascribed the cone-shaped structures to subaerial weathering⁷.

We propose that the Big Boulder Bed is an impact ejecta blanket proximal to a large, complex impact crater lying to the south of the western half of Cuba. This

location for the K/T crater is ~ 1,350 km from the site proposed by Hildebrand and Boynton² in the Colombian basin. This ejecta blanket was probably emplaced partially ballistically, and partially as a fluidized bed¹². The boulders of exotic lithology (target rocks) in the unit match the trace minerals found in K/T ejecta layers elsewhere in the world¹³. The cone-shaped concretions in the underlying sandstone might be either shatter cones caused by the impact, or fluid escape conduits.

Faults paralleling the southern coast of western Cuba¹⁴ could represent ring faulting associated with complex crater development and modification¹². A strong positive gravity anomaly south of the ring faulting and centred on the present coast may be due to impact melt sheets emplaced during crater formation. We note further that the Isle of Pines off the south coast of western Cuba, composed of schist and marble, might represent a central uplift feature; an impact centred here would have formed a crater ~225 km in diameter. Alternatively, the Isle of Pines may be only part of a multiple ring complex¹⁵, allowing for a larger impact structure whose radius is constrained only by the curved pre-Tertiary bedrock arc of western Cuba. Further onshore and oceanographic field work will be needed to confirm details of the location, size

and effects of the impact.

Note added in proof: Hildebrand and Boynton have just reported their discovery of shocked quartz grains in the Haitian K/T boundary layer¹⁵.

BRUCE F. BOHOR

US Geological Survey,
Box 25046, MS 972,
Denver,
Colorado 80225,
USA

RUSSELL SEITZ

11 Kennedy Road,
Cambridge,
Massachusetts 02138,
USA

1. Pszczolkowski, A. *Bull. Pol. Acad. Sci. (Earth Sci.)* **34**, 81–94 (1986).
2. Hildebrand, A. & Boynton, W. *Geol. Soc. Am. Abstr. Progr.* **21**, A371 (1989).
3. Maurrasse, F., Pierre-Louis, F. & Rigaud, J. J.-G. *Proc. 4th Latin Am. geol. Congr., Trinidad and Tobago* 328–338 (1979).
4. Maurrasse, F. *Geol. Soc. Am. Abstr. Progr.* **18**, 686 (1986).
5. Bohor, B.F. & Izett, G.A. *Proc. Lunar Planet. Sci.* **17**, 68–69 (1986).
6. Seitz, R. & Bohor, B.F. *Naturwissenschaften* **75**, 307–308 (1988).
7. Bohor, B.F. *Eos* **69**, 1291 (1988).
8. Palmer, R. J. *Geol.* **42**, 123–145 (1934).
9. Palmer, R. J. *Geol.* **53**, 1–34 (1945).
10. Neff, C.H. *Bull. Am. Ass. Petrol. Geol.* **55**, 1418–1482 (1971).
11. Pszczolkowski, A. *Bull. Pol. Acad. Sci. (Earth Sci.)* **19**, 249–259 (1971).
12. Melosh, H.J. *Impact Cratering: A Geologic Process* (Oxford-Clarendon, New York, 1989).
13. Bohor, B.F., Foord, E.E. & Betterton, W.J. *Meteoritics* **23**, 258–259 (1988).
14. Furrzola-Bermudez, G. *et al. Geology of Cuba* Translations on Cuba no. 311 (F.S. Dept of Commerce, Washington, 1965).
15. Hildebrand, A. & Boynton, W. *Lunar planet. Sci.* **21**, 512–513 (1990).

Opening plant calcium channels

SIR—Vesicle preparations of the vacuolar membrane (tonoplast) of plant cells, like those of the endoplasmic reticulum of animal cells, exhibit inositol 1,4,5-trisphosphate (InsP₃)-gated Ca²⁺ release^{1,2}. But even in membrane preparations highly enriched for tonoplast, the total extent of Ca²⁺ release never exceeds 30 per cent, and is frequently less than 20 per cent, of the non-bound Ca²⁺. This limited Ca²⁺ release raises the question of whether the vesicle preparations comprise sub-populations of membranes of variable intracellular origin, as appears to be the case from analogous studies on animal cells³.

The issue may have been neatly resolved by the recent patch clamp analysis of InsP₃-elicited currents in red beet vacuoles⁴. A comparison of microscopic (single channel) and macroscopic (membrane) currents enabled the author of that study to estimate the density of InsP₃-gated channels as about 1,200 channels per vacuole of mean radius (r_{vac}) = 23 µm. The mean radius of tonoplast vesicles (r_{ves}) prepared by density gradient centrifugation is about 1 per cent of this value⁵, and the probability that a given vesicle contains an InsP₃-gated channel is therefore $1,200 \times (r_{ves}/r_{vac})^2$, or only 0.12. Thus, the finding

that plant tonoplast preparations exhibit only limited Ca²⁺ release can be explained entirely on the basis of the relatively high surface/volume ratio of the vesicles in comparison with the parent organelle.

JAMES M. BROSNAN

Biology Department,
University of York,
Heslington, York YO1 5DD, UK

1. Schumaker, K.S. & Sze, H. *J. biol. Chem.* **262**, 3944–3946 (1987).
2. Brosnan, J.M. & Sanders, D. *FEBS Lett.* **260**, 70–72 (1990).
3. Thevenod, F. *et al. J. membr. Biol.* **109**, 173–186 (1989).
4. Alexandre, J., Lassalles, J.P. & Kado, R.T. *Nature* **343**, 567–570 (1990).
5. Pope, A.J. thesis, Univ. York (1989).

SIR—We wish to draw attention to the significance of the recent report¹ that inositol 1,4,5-trisphosphate (InsP₃) opens Ca²⁺ channels in the vacuolar membrane (tonoplast) of higher plants. This finding has broad implications for the regulation of ion transport at this membrane during signal transduction.

The dominant ionic channel in the tonoplast, the slow vacuolar (SV) channel² is weakly selective for cations rather than anions, and is activated by Ca²⁺ ions on the cytosolic side over a concentration range

indicative of physiological control. But the SV channel is also strongly inwardly-rectifying, and effects of Ca^{2+} have been observed only at negative membrane potential (V_m). Because, in the steady state, tonoplast V_m is sustained positive by the electrogenic H^+ pumps^{3,4}, the physiological role of Ca^{2+} in activating the SV channel has, hitherto, been doubtful.

The equilibrium potential for Ca^{2+} across the tonoplast is greater than -200 mV^{5,6}. Opening of Ca^{2+} channels during a transient rise in InsP_3 (generated, for example, by hormones⁷, light⁸ or elicitors) can therefore be expected to induce a negative swing of V_m . Because cytosolic free Ca^{2+} will rise simultaneously, the net effect will be a massive increase in SV channel activity.

These considerations clearly indicate a central role for the SV channel in regulating solute fluxes across the tonoplast of cells whose volume habitually changes significantly. In the specific case of stomata, where guard-cell volume reduction is known to be preceded by a rise in

cytosolic free Ca^{2+} (ref. 9), coordinated release of ions can be achieved both at the tonoplast and (via Ca^{2+} gating of other ion channels¹⁰) at the plasma membrane.

DALE SANDERS
EVA JOHANNES

Biology Department,
University of York, Heslington,
York YO1 5DD, UK

RAINER HEDRICH

Pflanzenphysiologisches Institut,
Universität Göttingen,
3400 Göttingen, FRG

- Alexandre, J. & Lassalles, J.P. & Kado, R.T. *Nature* **343**, 567–570 (1990).
- Hedrich, R. & Neher, E. *Nature* **329**, 833–836 (1987).
- Rea, P.A. & Sanders, D. *Physiol. Plant.* **71**, 131–141 (1987).
- Hedrich, R., Kurkdjian, A., Guern, J. & Flüge U.I. *EMBO J.* **8**, 2835–2841 (1989).
- Miller, A.J. & Sanders, D. *Nature* **326**, 397–401 (1987).
- Felle, H. *Planta* **174**, 495–499 (1988).
- Ettlinger, C. & Lehle, L. *Nature* **331**, 176–178 (1988).
- Morse, M.J., Crain, R.C. & Satter, R.L. *Proc. natn. Acad. Sci. U.S.A.* **84**, 7075–7078 (1987).
- McAinsh, M.R., Brownlee, C. & Hetherington, A.M. *Nature* **343**, 186–188 (1990).
- Hedrich, R. & Schroeder, J. I. A. *Rev. Plant Physiol. Plant molec. Biol.* **40**, 539–569 (1989).

Kinase and neurotransmitters

SIR—We have recently isolated¹ acidic proteins of relative molecular mass 29,000–33,000 from sheep brain that are potent inhibitors of Ca^{2+} -phospholipid-dependent protein kinase C. A search of the EMBL database reveals that peptides representing more than 90 per cent of the kinase C inhibitor isoforms are very similar to bovine brain 14-3-3 protein² (see figure). No physiological function had been attributed to this protein

until Yamauchi *et al.*³ showed that it is identical to an activator protein of tyrosine and tryptophan hydroxylases, the rate-limiting enzymes involved in catecholamine and serotonin biosynthesis respectively, processes essential for the synthesis of dopamine and other neurotransmitters.

Examination of the amino-acid sequences in the figure suggests a possible mechanism for kinase C inhibition.

	1	20	40	60
14-3-3	MGDREQLQRARLAEQAERYDDMASAMKAVTELNEPLNSNEDRDLSSVAYKNVVGARRSSWRV			
KCIP	MDDREDLVYQAKLAEQAERYDEMVSMAKAVTELNEPLTNEPRLSSVAYKNVVGARRSSNRV			
				RKGLRQK
Protein kinase C pseudosubstrate site				
	80	100	120	
14-3-3	ISSIEQKTMADGNEKKLEKVKAYREKIEKELETVNCNDVLALLDKFLIKNCNDFQYESKVFYL			
KCIP	ISSIEQKTMADGNEQNLPKSNEDRNMVETELKLISNDILDVLDKHLIPAANTG--ESKVFY			
	140	160	180	
14-3-3	KMKGDYYRYLAEVASGEKKNSVVEASEAAYKEAFEISKEHMQPHTPIRLGLALNFSVFYYEI			
KCIP	KMKGDYYRYLAEVAAGDDKKGIVDQSQQAYQEAFFEINKEHMQPHTPIRLGLALNFSVFYYEI			
	180	200	220	240
Lipocortin C-terminus	KGDIYEKALLALCGGDD			
	S K V VKI E			
	R I L N			
	T			
14-3-3	QNAPEQACLLAKQAFDDAIAELDTLNEDSYKDSSTLIMQLLRDNLTLWTSDQQDEEAGEGN			
KCIP	LNNPVK AAFDEAIAELDTLNESYQDSSTLIMQLLRDNLTL			

Alignment of bovine brain 14-3-3 (η chain²) with peptides from kinase C inhibitor protein (KCIP) in single-letter code. Three main isoforms of the kinase inhibitor have been partially sequenced and although only one set of representative peptides is shown, the overall identity is approximately the same, 75 per cent. The additional alignment at positions 52–59 is the pseudosubstrate domain of α , β and γ forms of protein kinase C^{4,5}. The 'pseudosubstrate' alanine residue is marked with an asterisk. Residues occurring in at least two out of ten lipocortins⁶ are also indicated. A good alignment could not be obtained for some kinase C inhibitor peptides with distinctly different sequence, in the region between residues 191 and the C terminus of 14-3-3.

Residues 54–57 in the 14-3-3 protein isoform and all the kinase C inhibitor forms we have sequenced are reminiscent of part of the 'pseudosubstrate' domain of protein kinase C^{4,5}. The pseudosubstrate hypothesis has been proposed to account for the inhibitory sequences in the regulatory domains of a wide range of second messenger-dependent protein kinases^{4,5}. These sequences contain all the elements necessary for a recognition site for the particular kinase but the phosphorylatable serine or threonine is replaced by another residue (most commonly alanine). Competitive inhibition by this domain is relieved by the conformational change induced by binding of the second messenger. Note that the serines at positions 58 and 59 are potential substrate sites for kinase C whereas cyclic AMP-dependent and Ca^{2+} -calmodulin kinase II could phosphorylate Ser 59.

Although the kinase inhibitor and 14-3-3 are not calcium-binding proteins^{1,6} and there is no evidence of similarity with any putative calcium binding domains, one region (residues 127–142) is very similar to the conserved carboxy terminus of the family of calcium- and lipid-binding proteins, variously called lipocortins, endonexins and calpactins⁷.

The proteins 14-3-3 and kinase C inhibitor exist as at least seven distinct gene products, but it is not known whether different isoforms are responsible for the physiological roles of this novel multigene family.

ALASTAIR AITKEN
CHRISTINE A. ELLIS*
ALAN HARRIS
LYNDA A. SELLERS
ALEX TOKER

Laboratory of Protein Structure,
National Institute for Medical Research,
The Ridgeway, Mill Hill,
London NW7 1AA, UK

*Mount Sinai Hospital Research
Institute,
Toronto,
Ontario M5G 1X5, Canada

- Toker, A., Ellis, C.A., Sellers, L. & Aitken, A. *Eur. J. Biochem.* (in the press).
- Ichimura, T. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 7084–7088 (1988).
- Yamauchi, T., Nakata, H. & Fujisawa, H. *J. Biol. Chem.* **256**, 5404–5409 (1981).
- Hardie, D.G. *Nature* **335**, 592–593 (1988).
- Parker, P. *et al. Molec. cell. Endocr.* **65**, 1–11 (1989).
- Boston, P.F., Jackson, P. & Thompson, R.J. *J. Neurochem.* **38**, 1475–1482 (1982).
- Pepinsky, R.B. *et al. J. Biol. Chem.* **263**, 10799–10811 (1988).

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of fewer than 500 words and five references. □

Gadolinium and friends

R. J. P. Williams

Biochemistry of the Lanthanides By C. H. Evans. Plenum: 1990. Pp. 444. \$95.40.

THIS book has a somewhat strange title. If one thinks of a book on the biochemistry of an element, copper for example, then there lies behind this title the presumption that copper has a biological function. The lanthanide ions have no known biological function. It is a further remarkable fact that the group of the periodic table to which the lanthanides belong, IIIA, has only one representative with a biological function, boron, and even its role is not known to be of significance in animals. All the other elements of the group give trivalent cations in solution, M^{3+} , as the lanthanides do, and are either innocuous or considered to be poisonous, for example Al^{3+} . It hardly seems as if a book on lanthanide biochemistry is warranted.

There are two good reasons why this is not so. First, the author shows the way in which experimentalists can use the prop-

erties of lanthanides to understand the functions of other elements in biology which have similar properties. The chemistry of elements in the periodic table shows that as well as relationships within groups, there are some diagonal relationships between neighbouring groups. Li^+ has somewhat similar chemistry to Mg^{2+} , Be^{2+} to Al^{3+} , and in biological systems Ba^{2+} blocks K^+ channels. The underlying reason for the diagonal relationship is ionic size. The lanthanides are close in size to Ca^{2+} . Thus we can make use of their properties to undercover features of biological interest, especially of Ca^{2+} . Second, it may be that the introduction of lanthanides into biological systems will lead to useful drugs, much as lithium is used. Lanthanides antagonize some functions of both calcium and magnesium.

Evans leads the way carefully into both fields, devoting much of the first six

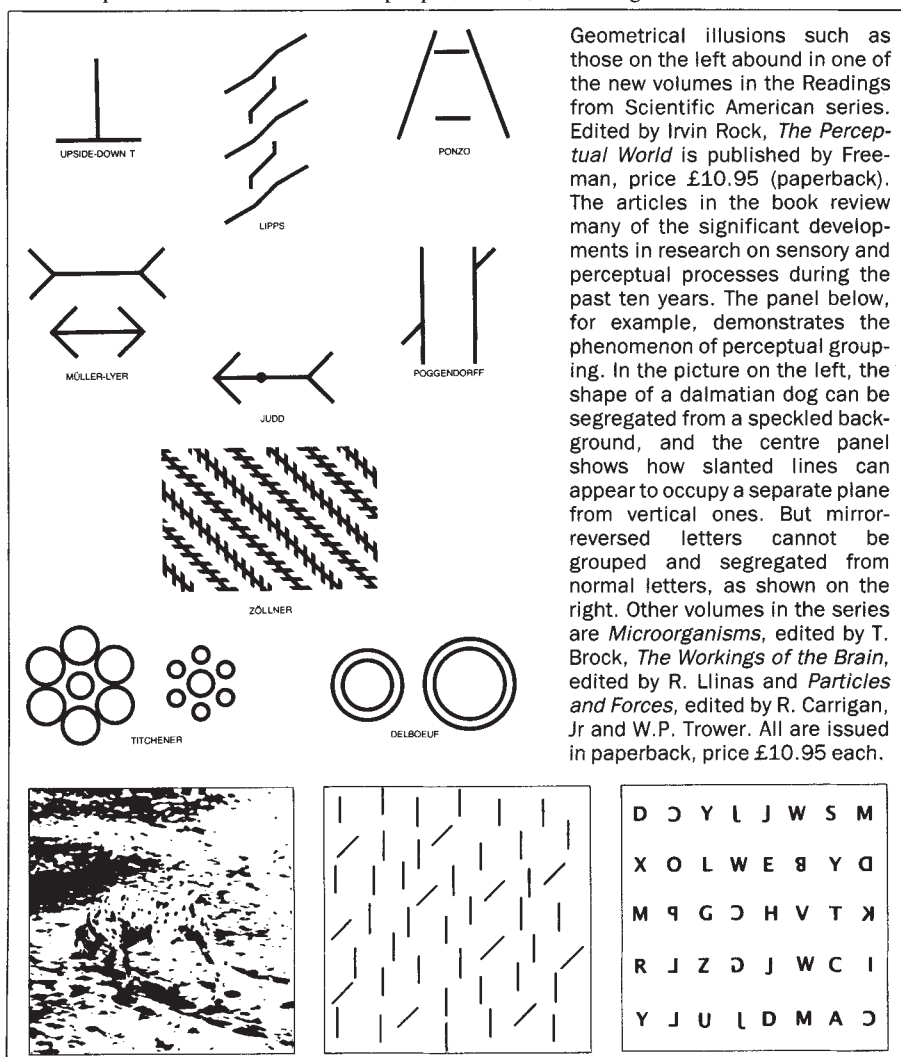
chapters to the physics and chemistry of lanthanide interactions with biologically interesting molecules and even cells. The essence of their value is that given the choice of about fourteen elements — the whole lanthanide series which is a 'transition' metal series — almost every physical technique can be used in association with their introduction. Lanthanides are then invaluable as probes of molecules, membranes and even cellular states, always as substitutes for Ca^{2+} . Some of the prettiest work illustrated here involves fluorescence and nuclear magnetic resonance studies of proteins. Of course, lanthanides can also be used as heavy atoms in electron-microscope stains or in X-ray diffraction studies.

Probing the properties of a biological site does not just involve spectroscopy or structure. We would like to understand the full power of biological selectivity of binding, so-called molecular recognition. No better opportunity is given to the researcher to understand the power of simple electrostatic recognition factors in aqueous solution than the analysis of binding of lanthanide ions to organic surfaces. In the series from La^{3+} to Lu^{3+} there is a change of ionic radius over a range of about 0.15 angstroms in steady increments of about 0.01 angstroms. If ever there was an opportunity for theorists to have an ideal test for their understanding of electrostatics, then this must be it. As Evans shows, there are plenty of data but very few attempts to explain the results of studies of these inorganic site-specific reagents. An outstanding problem, as always, is hydration of the free and bound states.

The use of lanthanides as drugs is considered in the later part of the book and testings are described at some length. The feeling grows on reading that the problem is a matter of delivery of the lanthanides to the right place, for there is no doubt that there are effects that could be useful not just on enzymes, pumps and channels, but also on bone. The only considerable value of the lanthanides in medicine to date is their use as contrast agents in nuclear magnetic resonance scanning, in which context gadolinium is the most powerful ion.

Evans has written a well-balanced book. I am left with the impression that lanthanides will reveal a great deal about the way calcium acts and the theory of electrostatic binding, for example, but also that we are missing a trick in application. The use of inorganic drugs has only started on a long trek of exploration, and by the time I finished this book I had the distinct feeling that the lanthanides have considerable potential in biochemistry. □

R. J. P. Williams is in the Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QR, UK.



Troublesome relationships

David Dean

The Human Career: Human Biological and Cultural Origins. By Richard G. Klein. University of Chicago Press: 1989. Pp. 524. £31.95, \$45.95.

The Hominid Gang: Behind the Scenes in the Search for Human Origins. By Delta Willis. Viking: 1989. Pp. 352. \$19.95.

DURING the past decade, most teachers of undergraduate courses in palaeoanthropology have used Wolpoff's comprehensive, but now dated, text *Paleoanthropology* (Knopf, 1980). *The Human Career* is likely to take over as the 'mainstream' textbook of the early 1990s.

Klein, a Plio-Pleistocene archaeologist, is in a unique position to write on human evolution. He is not in the middle of the taxonomic battles that have dominated other textbooks, often at the expense of reporting important archaeology debates. Klein's inclusion of archaeological and geological evidence gives palaeoanthropology its fullest range and depth.

The section on primate ancestors covers the debates on the primate petrosal bulla, the catarrhine status of *Amphipithecus* and *Pondaungia*, the relationship of the Fayum taxa to later catarrhines, platyrrhine origins and the diversity of Miocene apes. But a teacher using this book would need a fuller discussion of Oligocene and Miocene catarrhines. Again, despite a thorough discussion of the main dating methods, the issue of molecular clocks is mentioned all too briefly.

In his discussion of the stratigraphy and dating of the African australopithecine and early *Homo* sites, Klein presents the morphology of important specimens in accurate illustrations adapted from well-known publications. He provides a framework and discusses the main controversies: gracile versus robust, bipedalism, how many species, hunting versus scavenging, and so on. Not unexpectedly, Klein concludes that Oldowan peoples were "probably far less bloodthirsty than popular authors such as Ardrey (1961, 1976) have imagined".

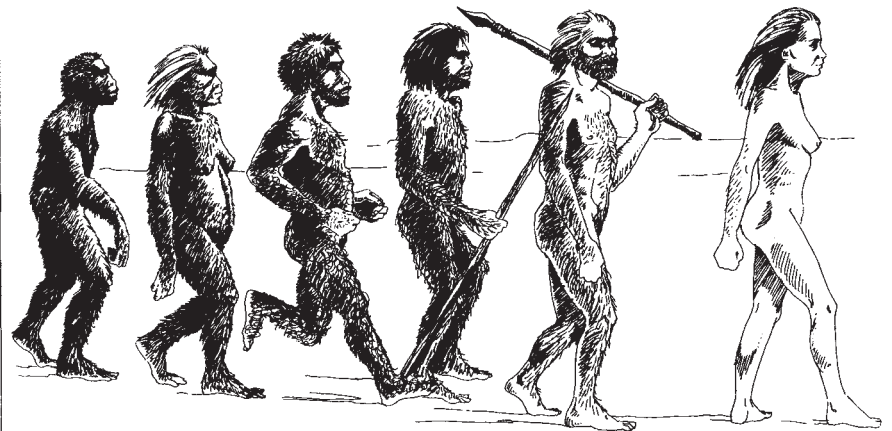
Klein presents the history of the discoveries of *Homo erectus* by Dubois, Black, Weidenreich, von Koenigswald and later workers in masterly fashion. Issues such as the current disagreement over whether the Far Eastern specimens represent an evolutionary dead-end or show regional continuity with populations living in those areas today are also covered. In the case of the dead-end hypothesis, few would argue with Klein that "the validity of this hypothesis [is] far more important than finding a precise

taxonomic assignment for key European, African, and Asian fossils". Klein's presentation is only slightly behind in covering the current debate on the reality of the Movius line and the dating of the earliest Far Eastern sites, but these issues are often ignored in other textbooks.

Klein makes a strong case for the origin of earliest *H. sapiens* as a glacial Middle Pleistocene population that became adapted to either a cold Europe or a dry Africa, together with the mainstream view that no European *H. erectus* is yet documented. Klein subscribes to the traditional view that all the European fossils older than 130,000 years are archaic *H.*

meetings she attended over the past 12 years, as well as on several trips to Africa. With a reporter's knack, she recounts conversations, debates and personal rivalries among the 'heavyweights'.

Unfortunately, her presentation of the facts is such that I would not recommend this book to the lay public at which it is aimed. Willis misuses taxonomic terms and common names alike. She says the sample of Kenyan *Australopithecus* includes "robust and hyper-robust (or *boisei*)". But *A. robustus* is only known from South Africa. Constant reference to *afarensis* (not *A. afarensis*) implies to lay readers that species are correctly referred



A standard illustration seen on museum walls and in school textbooks, these reconstructions suggest a far more concrete knowledge of human evolution than we actually possess.

sapiens; but he adds that it "seems reasonable to hypothesize an evolutionary sequence from the earlier group [ca. 500,000–200,000 years] to the later one [ca. 200,000–130,000 years] to the Neanderthals".

Neanderthals are presented as "the last truly 'primitive' human group". Late archaic specimens and stone-tool industries from outside Europe are also detailed. Klein then presents exhaustive evidence that modern human populations appeared some time between 250,000 and 50,000 years ago, probably first in Africa more than 90,000 years ago. Klein is equivocal on the fate of the Neanderthals: "Increasingly, the answer seems to be that they were physically usurped, but the issue remains debatable."

In making the case for Cro-Magnon superiority over both Neanderthals and their early anatomically modern brethren, Klein seems to equate European Aurignacian tools with modern behaviour: "... in both the Near East and Africa, anatomical modernity antedates behavioural modernity ... Neurologically, they may have lacked the fully modern capacity for culture."

Willis's *Hominid Gang* is the group of Kenya Museum staff that searches for fossils. The subtitle of the book promises gossip, mood lighting and piquant vignettes, all of which are delivered. Willis reports on the palaeoanthropological

to by their trivial names alone.

Willis also confuses simple facts. More than once she says hominoids have a V-shaped jaw. This is correct for lower primates, but ape mandibles are most often described as U-shaped and hominid mandibles as "parabolic". She claims: "*Erectus* skulls found in Asia have a sagittal crest, but this is absent from some *erectus* skulls found in Europe and Africa. ... *Erectus* skulls have a bony bump at the base of the skull called an occipital bun (as in a lady's hair bun)." But there is no widely acknowledged *H. erectus* in Europe, nor do any *H. erectus* specimens exhibit a sagittal crest (as do robust australopithecines or gorillas) or an occipital bun (as do Neanderthals *sensu stricto*). There is also a strange reference to 'three lemons': "This is an eye-eye; those are two mouse types" (p. 291). This might lead readers to assume that the aye-aye is the only stereoscopic primate.

Taken as introductory-level texts, *The Human Career* is successful whereas *The Hominid Gang* isn't. Palaeoanthropology is not a soft, descriptive science as some would believe. Organization and fact-checking are as important to its reportage as is testing of hypotheses to its praxis. □

David Dean is in the Department of Anthropology, City University of New York, New York, New York 10036-8099, USA.

From *The Search for Eve* by M. H. Brown (Harper & Row).

Fruits of a treasure hunt

Michael Usher

Mathematical Modeling in Ecology. By Clark Jeffries. *Birkhäuser*: 1989. Pp.193. £27.50, \$29.95.

Matrix Population Models. By Hal Caswell. *Sinauer*: 1989. Pp.328. Hbk \$50, £40; pbk \$28.95, £23.95.

THESE two books fit firmly into the quantitative wing of the science of ecology: one is concerned with mathematical modelling in general and the other with the applications of a particular form of models into a single branch of ecology — population biology. Jeffries has written an introductory text, explaining the mathematics of a few deterministic models, the results of these models, and then looking for similar patterns and relationships in nature. The approach of the book is therefore not one of "here's an interesting biological phenomenon; let's explore it with a model". Caswell starts from this latter perspective; his book is firmly rooted in demography and he develops a class of mathematical models to analyse and interpret the ecological phenomena observed.

Jeffries uses some simple concepts in his introduction. Using a grid of x, y coordinates, he introduces the concept of a treasure hunt with clues attached to each point indicating the direction of the next move. The list of locations and clues may look daunting, but if a student takes a few minutes to plot points and arrows on a sheet of paper, all is simplified so that, wherever one starts, one ends up at the central location. This simple example nicely introduces the concept of trajectories in two-dimensional difference equations. I did not find it useful to have a program for a Hewlett Packard 41CV calculator to handle such equations (contained in an appendix to the chapter), but I do complement the author on providing a series of problems with their appropriate answers.

Jeffries' book is short and covers only a handful of the topics that could have been included. The subjects introduced explore some aspects of ecosystem models, stability, aspects of attractor regions and the flow of energy through a system. As the treatment is from mathematics to biology, at times I felt that the links were forced. In general, the explanations are well done and the models well-introduced, but the student needs to beware of typographical errors, especially related to the "dot" notation for the derivative (for example on page 68 the statement that "if $x=K$ then $\dot{x}=0$ ", when the latter should have been $\dot{x}=0$).

Since the publications of H. Bernardelli, E. G. Lewis and P. H. Leslie in the 1940s, there has been much interest in the use of matrix models for the analysis of popu-

lations. These models were first used for age-structured populations, leading to the realization that in nature both mortality and fecundity are age dependent. In the 1960s the models were modified to incorporate size- or (for insects) stage-dependency. The number of papers addressing these models has increased year by year. They are introduced more frequently into the basic ecological textbooks, but this is the first book to be devoted solely to their study.

Caswell introduces his subject via the more traditional life-table approach but also uses examples to illustrate birth-flows, birth-pulses and survivorship. In the early chapters he balances the age- and stage-structured approaches to building these matrix models, before progressing to use the models for population projections (using eigenvalues and eigenvectors). In the later chapters, Caswell explores a whole variety of topics, including confidence limits on the eigenvalue, the incorporation of males into the model, stability of the model, stochasticity of the environment and the inevitable modern topics of bifurcations and chaos. Caswell concludes with an appendix on basic matrix algebra — but should this have come first? Unlike the brief index of Jeffries' book, there is a full index in Caswell's.

I feel sure that Caswell's book will be satisfying for the research ecologist. Although the subject matter is superficially narrow, the approach is explorative. The models are introduced, and many implications both of a theoretical, mathematical nature and for the theory of population biology are explored. It is not a particularly easy book to read, but well worth the effort. (If your matrix algebra is rusty, brush it up before attempting chapter 2.) Jeffries' book is not aimed at the researcher, but at the student wanting to gain an insight into some modelling approaches (but I suggest that the student also needs to revise some linear algebra, as well as calculus, before starting it). I did not derive the same sense of satisfaction from Jeffries' book as I did from Caswell's. I suppose that if mathematics and biology are going to interact, I prefer the mathematics to be derived to explain biological phenomena, not that biological examples are hunted down to support mathematical constructs. I remember the old advice of K. E. F. Watt, that models should be "steeped in the lore of biology". □

Michael B. Usher is in the Biology Department, University of York, York YO1 5DD, UK.

■ Another recently published volume on this subject is *Introduction to Theoretical Ecology* by Peter Yodzis. The book is a collection of lecture notes for students of ecology, and covers theories of population, community and life-history, developing mathematical models for selected topics. The publisher is Harper and Row, price £33.95, \$48.50.

■ Harper and Row are also the publishers of *Ecological Methodology* by Charles J. Krebs. In this book, the author presents the statistical methodology general to ecological field measurements. Price is £33.



Completely batty — grey long-eared bats (*Plecotus austriacus*) are one of Britain's 15 resident bat species that appear in *A Field Guide to British Bats* by F. Greenaway and T. Huston. The book contains 40 colour photographs taken by Greenaway of the Natural History Museum in London, and the text is written by Huston, Britain's bat conservation officer for the past six years. Published tomorrow by Bruce Coleman, 17 Windsor Street, Uxbridge UB8 1AB, price £10.99.

Letting the bald patches show

Frederick Urbach

Ozone Depletion: Health and Environmental Consequences. Edited by Robin Russell Jones and Tom Wigley. Wiley: 1989. Pp.280. £35, \$72.

IN THE spring of 1971, it was suggested that the exhaust gases of a future fleet of supersonic aircraft might have an effect

the British Antarctic Survey, beginning in 1975 and intensifying thereafter, led to a concerted international research effort in 1987 which, for the first time, clearly demonstrated that chlorofluorocarbon (CFC)-derived chlorine atoms are one of the chief causes of ozone destruction. This volume represents the edited proceedings of a conference held in London in November 1988, at which scientific and medical authorities as well as politicians and environmental activists addressed a wide range of subjects: ozone depletion, global warming, international controls, ultraviolet-induced carcinogenesis, global

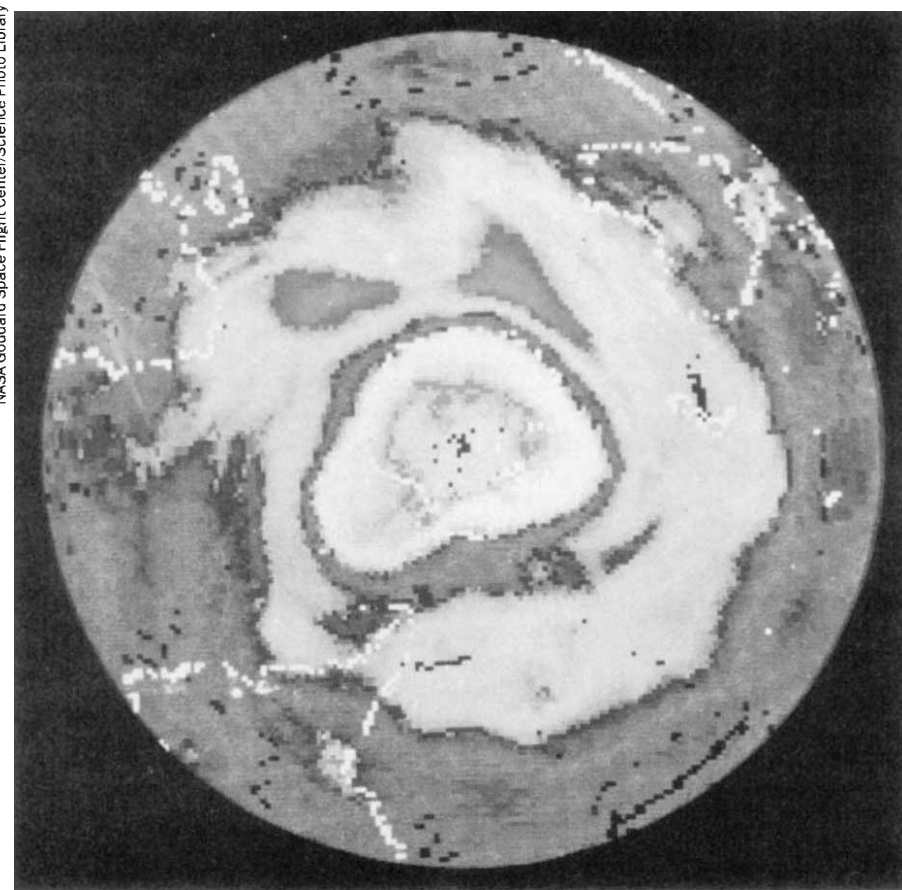
cause 15–20 per cent of the expected warming. Furthermore, changes in atmospheric temperature may lessen the effect of CFCs on ozone. It is difficult to derive models projecting the degree of future warming because the variables, such as the effect of oceanic thermal inertia are unknown — but everyone agrees that warming is to be expected.

The political ramifications of international controls of greenhouse gases, including CFCs and halons, are global, because control can be effective only if all the main producers and consumers are involved. A significant beginning has been made with the Vienna convention in 1985 and the Montreal protocol in 1987. But in view of more recent observations, attempts should be made to speed up implementation of these agreements.

One of the main potential problems to humans of increased ultraviolet radiation is the increase in skin cancers via damage to DNA, which if misrepaired can cause skin-cell carcinogenesis. Ozone depletion in the stratosphere could seriously increase the incidence of malignant melanoma, the most dangerous type of skin cancer, but details of this relationship are not yet clear. Other potential consequences of ozone decrease are the effects on terrestrial plants and marine organisms. Effects on the food chain are serious, but the confidence and precision of estimates vary greatly. Considerable research is needed to understand these relationships.

Some contributors to this volume present impassioned pleas for the development of alternatives to CFCs, but others stress the enormous technical and economic costs involved. The book contains reasoned and realistic views both of concern for environmental problems and of the difficulty of having nations act in concert. The scientific sections are excellent and up to date, providing a brief but reasonable review of the state of the art in 1988. □

Frederick Urbach is at the Temple University Medical Practice, Fort Washington, Pennsylvania 19122, USA.



This satellite map, compiled from data obtained by the Nimbus-7 weather satellite, shows the severe ozone depletion over Antarctica (2 October 1987). The ozone 'hole' is the central white outlined area. Australia, southern Africa and part of South America are also outlined.

on stratospheric ozone content. It was thought that such an effect would be caused by water and nitrogen oxides emitted as combustion products of aircraft engines operating in the stratosphere. In 1974, Molina and Rowland suggested that ozone would be destroyed by catalysis of chlorine atoms and that the main anthropogenic source of chlorine atoms could be chlorofluoromethanes. In the next decade, much laboratory and field research strengthened this supposition and led to heightened public awareness of the dangers of increased ultraviolet radiation on living organisms — including, of course, humans.

The springtime decreases of stratospheric ozone over Antarctica reported by

consequences and political aspects.

The resulting book provides an overview of our knowledge of stratospheric ozone chemistry and the sources of active molecular species acting as catalysts in ozone destruction. The problems of modelling stratospheric ozone perturbation and of estimating changes far into the future are difficult ones. There is agreement that ozone will decrease in the stratosphere and increase in the troposphere, and that long-term, continuous calibrated datasets are needed to understand global-scale changes.

The rationale for discussing global warming in this context is that the CFCs implicated in ozone reduction are also potent greenhouse gases, estimated to

Corrections

■ In Stuart Sutherland's review of *Microcognition* by Andy Clark (*Nature* 344, 207; 15 March 1990), the title of the book was misspelled.

■ *Primate Origins and Evolution* by R. D. Martin, reviewed by Jonathan Marks in *Nature* 344, 205; 15 March 1990, is published in the United States by Princeton University Press.

■ In his review (*Nature* 344, 301; 22 March 1990) of *A Shield in Space?* by S. Lakoff and H. F. York, Richard Garwin cited a two-year-old estimate of \$20,000 for the cost of a 'brilliant pebble'. This figure should have been \$50,000 per unlaunched pebble and \$25,000–\$50,000 for launch into orbit. Thus the unlaunched cost estimate of a brilliant pebble has changed from \$50,000 two years ago to \$1.1 million–\$1.4 million today. □

The great European experiment

In science, as in much else, the newly democratized countries of Europe will have much to offer. But the way will be hard.

THE survey which follows of the state of science in Eastern Europe is inspired by a few simple considerations. First, the events of the past year have been of absorbing interest, and are likely to prove of lasting importance. Revolutions usually are. Second, four decades of lack of liberty notwithstanding, the scientific communities of many of the countries in this belt through central Europe are numerically strong and intellectually alert. What contribution will they make to general understanding in the decades ahead? Third, how are these still-isolated groups of people seeking to adjust their way of doing professional business to the new circumstances in which they find themselves? Perhaps there are lessons from elsewhere to be learned. Necessarily provisional though it is, it is hoped this survey may also assist in putting flesh on the bones of Eastern Europe's dream of "coming back to Europe".

Revolutions are political affairs, as are their consequences. There is no escape from that, which is why articles that follow refer at several points to the political aspirations of those who have lived through the revolutions in the past year — and to the political conflicts that persist. Readers in Eastern Europe will not be surprised. Those elsewhere who may be, perhaps on the grounds that science and politics have nothing to do with each other, should think again. Some in the West should also consider whether their past willingness to accept East European science on the terms on which it has been represented by now-discredited institutions has contributed to the plight from which Eastern Europe has yet to emerge.

Soviet compliance

From Poland in the north to Bulgaria in the south-east, the past year's upheaval has been made possible by the compliance, even the encouragement, of the new leadership of the Soviet Union's Communist Party. It has been driven by two forces: brave people's pursuit of personal liberty and more general envy of the prosperity of the West. The discredited governments of Eastern Europe were not classical tyrannies. They were dictatorships all right, and were also inefficient and corrupt. But they were dictatorships of the proletariat, in the sense defined by Marx, with the Stalinist twist that only members of the Party participated in the collective dictatorship. That is why local party cells had a licence to tell laboratory directors whom they should promote, or university authorities whom they should admit as students, while more exalted Party organs assumed the right to appoint the managers of public institutions — university rectors and deans, presidents of academies of science, for example.

Even so, the old governments of Eastern Europe would probably still be in power if they had been able to keep the promises they had made, mostly on the economic front. For doctrinal reasons, but with varying enthusiasm, they held to the correct principle that science and technology are the engines of technical innovation and, ultimately, of prosperity. There are more than 250,000 people in Eastern Europe who work professionally as scientists and technologists.

So what went wrong? The overarching error was the application of the untested doctrine that central planning is an efficient way of directing people's energy and of allocating resources. Fifty years of often bitter experience have shown that central planning, necessarily giving bureaucrats responsibility for decisions whose implications they cannot understand, is inefficient. Onlookers from the West should note that it is this system, not Communism in the abstract, that has now bitten the dust.

What is the alternative? Democracy is a part of every

answer. The unanimity would be more compelling if the concept were better understood. For the time being, it is taken to mean election, not merely of governments (which is fair), but of factory bosses, laboratory directors and of all those who hold important public offices or who are members of important advisory councils. The objective commands everybody's sympathy. Eastern Europe seeks to throw off the corrupt system of the *nomenklatura* — the office-holders appointed by Party officials behind closed doors for reasons not always apparent. It is crucial that the mechanism for electing governments should work fairly, but can it be wise that everybody else should run the gauntlet of his or her local ballot-box?

Seven countries in Eastern Europe also agree, with varying degrees of enthusiasm, on another common ingredient of a new recipe: the market economy. East Germany should get there soon by *Anschluss*, Hungary has been working towards the same end for a decade and Poland (whose farmers were never collectivized) has taken a huge and painful step towards opening its economic system to the West. But even the enthusiasts for these reforms do not fully understand what remains to be done. Free markets require people with a feeling for *östmärken*, *zloty*, *koruna* and *florints* and the rest in numbers that do not now exist, but also — because free labour markets entail at least temporary unemployment — social security systems to which very little thought has been given outside Hungary. Add to that the crying need for capital investment in the most rudimentary of productive enterprises and public services to conclude that the market economy is further away than most of Eastern Europe would like to think. The obvious danger is that the years ahead will bring disillusion before prosperity.

This question has an immediate bearing on the future of the scientific enterprise in Eastern Europe: on what scale will it be affordable? Nowhere has the question yet been asked as seriously as it is bound to be when democratic governments set about balancing their books. For many, the answers will be disappointing, perhaps cruelly. One of the benefits of a centrally planned economy is that questions such as these do not have to be asked. The bureaucrats decide how much of what there will be, and the resources flow accordingly. The danger, not yet fully appreciated, is that the



The seven countries covered here — with the 1914 divisions shown inset.

market economy which is an ingredient of the common dream will prevent even well-wishing governments from keeping research alive. Equipping laboratories to be competitive with those in the West is something else again.

That is why there is a certain unreality in the logic-chopping now under way throughout Eastern Europe about the means by which research funds should be allocated. Resentment of cronyism, and of the doctrine that established big-shots, have the first claim on what funds there may be, has bred the conviction that research funds should be awarded competitively. But how? On the model of the US National Science Foundation? Or Britain's research councils? France's CNRS? Italy's CNR? Both West Germany's Max-Planck Gesellschaft and DFG, or just one of them? Whatever the institutions, they will be irrelevant if there are no funds to spend.

Two common ambitions are nevertheless thoroughly to be applauded. First, it seems agreed that the gulf between universities and research institutes which has been a conspicuous feature of the past 40 years should be bridged, preferably by requiring researchers at both places to compete for the same funds. And the networks of research institutes controlled for 40 years by Stalinist academies of sciences should be more intelligently managed. Most researchers would prefer to stay within the framework of reformed academies, perhaps on the principle that the devil you know is safest. But that will usually be mistaken.

Academic matters

The place to start is with the academies; what, in post-revolutionary Eastern Europe, are they for? They have three functions, two of which are incompatible with each other and the third of which is irrelevant to present needs. Direct responsibility for the management of research is evidently in conflict with that for advising government (and the public generally) on technical matters. Which of Eastern Europe's academies will be the first to say that it manages too much research? Which (until now) has complained that its paymasters have stifled creativity by cramping able researchers' freedom? So, generally, the academies should become high-level sources of advice, some of it unwelcome. The restoration of the traditional honorific function of the national scientific academies could wait until the dust has settled (and the old guard has died off). The management of research should be some other organ's function.

Whose? The Stalinist way of running science is nothing if not comprehensive. Not only is there an academy of sciences but, usually, a state committee for science and technology — a kind of government department with a supraministerial coordinating function serving as a conduit for funds. Why not give the basic research institutes collectively the autonomy of a kind of Max-Planck Society, but make it plead annually for funds with a state committee also charged with saying how much should be spent on competitive research grants and on industrial development? The chief objection is that the state committees are citadels of the *nomenklatura*, but that is a poor defence. Why not just reform them? What else is a revolution for?

These are administrative matters. The people problem is more important and more serious. Eastern Europe is filled with able and imaginative scientists who are badly paid, but who are housed almost for nothing in what would be a sellers' market if apartment-sellers were encouraged. The result is that it is easier, and much more rewarding, for an able scientist to move abroad than to another laboratory in his home country or elsewhere in Eastern Europe. That is absurd when Eastern Europe's science managers wring their hands in fear that their best and brightest will forthwith decamp. The remedy is in their own (or their own governments') hands: reward established able people who choose to stay at home with an apartment they may legitimately sell on the housing market that must be created sooner rather than later. That would both reduce the pressure on salaries and abate the impending flight of able people. It would be a market solution, but what other kinds of solutions are there in a market economy?

A more serious people problem stems from the past year's up-

heavals. Revolutions put underdogs on top, discrediting those who are displaced. The result, now, is that senior or responsible positions must be filled either with able people who lack achievement and experience, perhaps by the denial of travel opportunities, or with experienced people who are tainted by past associations. It may be too much to hope that bitterness engendered by the recent past will be quickly subsumed by a prudent concern for the meritocracy, but the sooner past quarrels are buried, the better it will be for East European science.

What, in these circumstances, can the rest of the world do? Here is a simple shopping-list, culled from what people in Eastern Europe say, but aimed at those elsewhere who believe that science and scholarship are indivisible, and that the whole is greater than the sum of the parts:

Travel funds. The isolation of Eastern Europe is almost palpable: able and established people have been kept where they are for decades on political grounds, newly qualified scientists given a spell abroad are not then able to keep the links they make alive by revisiting. Few can attend conferences in their fields even when the venues are just a few hundred kilometres away. Raising the air fare in a local currency has not (until recently) been difficult, but paying for a taxi to the conference venue may be impossible for lack of hard currency. Eastern Europe badly needs a travel fund: \$10 million a year would make a huge difference.

Equipment and supplies. People with friends in the West have often been helped with crumbs from richer tables — a few milligrams of a special chemical here, a few transistors there. But even when (not often) there is hard currency, the local bureaucracy impedes access to essential supplies. Rarely can people jump quickly on a new bandwagon by telephoning a willing supplier. Often, approved supplies arrive only after 18 months, by which time the need may have disappeared. But those are the frills only. The near-lack of major items of modern laboratory equipment drives energetic people to suggest that collaboration with groups elsewhere may be the only sensible way of making progress. Some wonder (why not?) whether manufacturers might lend equipment in the hope of winning orders later.

Collaboration. These are some reasons why creative people throughout Eastern Europe are seeking collaborations with stronger groups elsewhere. They offer diligence, even donkeywork, in the pursuit of common goals.

Exchanges. Bilateral agreements with overseas institutions are valued wherever they exist, but are insufficient and insufficiently informal. Currency inconvertibility makes reciprocity necessary. (Some laboratory heads try to ensure that their exports to the West are not underpaid, not always successfully.) One unexploited resource is the capacity of Eastern Europe's universities to provide high-quality education (for undergraduates and graduates) at low cost.

Communications. The dearth of hard-currency books and journals is crippling. Books are worse than journals: people cannot expect to be given reprints of them for nothing. But even internationally known specialists find themselves without access to the journals dominating their fields. (Since the beginning of the year, only two libraries in Prague subscribe to *Nature*.) Will new technology help? Not much. Personal computers exist, but not commonly, modems are much more scarce and the quality of telephone transmission would, in any case, defeat them. But it is difficult to see why the West should not link one node in each of the Eastern European countries with the international academic data networks, letting them improvise their way from there. Some visionaries dream of when optical disk technology will make general access to journals affordable, but they know that decades will pass before that happens.

Culture. Eastern Europe is wedded more strongly than the West to the notion that science is a part of the general culture, and that the general culture deserves to be honoured for its own sake. That may have been Central Europe's distinctive contribution, a century ago, to the world we all now live in. Would it not be fun to bring back that lost tradition? □

Looking forward apprehensively

Warsaw

NAIVE expectations that people's spirits would permanently have been lifted by the election of last June, and by the formation of a government in which *Solidarity* is in a majority, are quickly dispelled: people look glum, and often talk as if they were. There are several explanations.

Nine months is a long time in quickly changing Eastern Europe, the euphoria of *Solidarity*'s election victory has been followed by the *Solidarity*-led government's deliberately engineered economic squeeze and nobody knows what the future holds. The economic reforms are an acknowledged gamble. The political

POLAND

Population: 37.8 million
Area: 312,683 sq. km
Per capita GNP: \$2,070
Higher education: Eleven universities and 81 other institutes. Total no. of students: 343,400.



future is in doubt: will *Solidarity* hang together? (Farmers and trade unionists have less in common now than a few months ago.) Will a political party that began life as a kind of trade union be able to stomach, let alone administer, the unemployment that economic reform

must eventually bring? And what will be the future course of relations with the Soviet Union?

Part of the trouble is that Poland has seen countless new dawns — not just liberation from the tsars in 1920, but the more recent phenomenon that each newly elected party leader would promise change, and would then renege. Poland would no doubt be even more apprehensive now if the past winter had not been unseasonably warm.

Meanwhile, the government is in a hurry. The speculation is that it is working towards another election about a year from now. The old Communist Party is still entrenched in a substantial minority of parliamentary seats, while the government is essentially a coalition of *Solidarity* and what used to be the Communist Party, members of which occupy the ministries of defence and interior as of right. This is part of the price Poland had to pay for being first with revolution, but the radicals look for electoral legitimacy.

Two educational reform bills were read for the first time in the Lower House of the Sejm (parliament) on 23 March, and may be law a few days from now. One will

Head start: the democratic reforms started by Lech Walesa and *Solidarity* gave Poland impetus for reform.

(among other things) guarantee the autonomy of the universities and other institutions of higher education, the other will regulate the granting of degrees. But the civil servants are already hard at work on two other measures — one to create a novel and competitive mechanism for awarding research funds in universities and public research institutes, the other to reform the Polish Academy of Sciences.

The rush to get these measures through Parliament is partly the government's wish to get something, almost anything, done. But even among the government's

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

supporters are some who believe that the reforms being introduced are insufficiently thorough, not to say radical, and may even be unworkable. And there is the fear that the new statute book will be uncomfortably permanent.

The sense of unease is everywhere coloured by the economic problems, from which there is no obvious escape. The causes are straightforward. The government has determined that Poland's future rests on normal economic relations with Western Europe, with the consequence that the larger part of its economy must be run on market lines. Already, for example, to ensure that agricultural exports keep flowing to the West, Poland has had to promise (the European Community in this case) that what it sells will not be subsidized.

The short-term discomforts are plain. The biggest step in that direction was the decree that, from 1 January, many prices would be calculated independently and many subsidies abolished. The immediate consequence was that average retail prices, for services as well as goods, increased by 79 per cent between last December and January. (The average increase of food prices over the month was 86 per cent.) But salaries, on average, increased by only a third at the beginning of the year, with the result that people's purchasing power declined by a quarter. Personal hardship is commonplace.

February's inflation, although lower, has tightened the screw still further. But by mid-March, the government was claiming success for its plan to give the economy a short sharp shock — the prices of many commodities had begun to fall as

ACADEMIC LIFE

Academics turn to government

Warsaw

THE most conspicuous contribution of Poland's academic establishment to the post-revolutionary period is that it has provided the small army of people who now run most of the government. The new law on the organization of science in Poland, for example, is the responsibility of Dr Stefan Amsterdamski, until a few months ago an academic lawyer at the University of Warsaw.

Dr Janusz L. Grzelak, now Deputy Minister of Higher Education, is similarly a psychologist saddled with ministerial responsibilities who reckons that his excellent spoken English was polished by a year spent in the Netherlands in 1986. (He regrets that it was not necessary to learn Dutch.) He is proud to have been a thorn in the flesh of the previous government during Martial Law, among other things by editing a monthly subversive journal.

Grzelak considers it part of Poland's strength that 70 per cent of its active scientists work in universities, and that many of them have been able to remain active in research. The practice of concentrating not simply support for, but the direction of, research in the Academy of Sciences has

not been followed as closely here as elsewhere in Eastern Europe.

The new education law will help, not least by its restoration of autonomy, by the removal of the influence of the Communist Party on the appointment of academics and even the selection of students and by the requirement that university posts should in future be filled by open competition. Nepotism will not be as easily sustained as in the past, while the old rigid hierarchy of progression up the academic ladder has been enormously simplified.

But there are problems. The new law gives equal status to 117 institutions of higher education, some of which are specialized schools, conservatoires and even a small school of theatre design, for example, while others have a more general pattern of education but "do not deserve the name" of universities. But Grzelak resists the view of some academics that the new arrangements will mean that there are too many people competing for the same research support.

But of one thing, Grzelak is certain. Will there be enough money to run the system now being designed successfully? "I can tell you right now. No!"

J.M.

retailers learned that they had to reduce their margins to persuade money out of people's almost empty pockets. The result, now, is that there is plenty in the shops, but a shortage of customers. The economy seems to be heading for a recession, which will give the country its first taste of unemployment for a long time. Poland has set about learning the law of supply and demand the hard way.

The currency unit, the zloty, has also been devalued to a level at which it is almost feasible to use it as a convertible currency. (US\$1 is now worth about 10,000 zloty.) The results are often bizarre. One laboratory director who had

ordered a sizeable batch of reagents from the West towards the end of last year found himself faced with a bill for nearly 500 million zloty in January. Happenings like that should quickly reduce the demand for imports, but at great cost to those whose work depends on them.

The short-term problem is that of the hardship and dislocation caused by inflation on such a scale. Further ahead lies the question of how Poland will eventually make its way in the world. Most people seem confident that "it'll be all right in ten years". Their problem is that there is no obvious way of getting from here to there.

J.M.

Eager to grasp autonomy

Warsaw

THE Polytechnic University of Warsaw, Poland's MIT, is sited on a city-centre block one must enter as if into an opera house. First, there is an entry-way, with brass-handled swing doors, a cavernous congregation-space with stairways leading off in all directions and then the discovery that all the stairways lead into or alongside a five-storey atrium with the floor-plan of a heptagon with a single axis of symmetry but otherwise unequal sides. There is no proscenium, of course, but instead an almost moorish wedding-cake structure that might have been designed for a performance of Aida. One day at the end of last month, a beginners' surveying class was hard at work with theodolites on the ground floor, no doubt plotting its shape for the umpteenth time.

What will post-revolutionary freedom, and the new law on higher education in particular, do for the polytechnic university? Rector Marek Roman, while the new law was still before the *Sjem*, had one ingenious scheme up his sleeve. Polish institutions of higher education provide students with meals and housing at subsidized prices, with funds provided by the state, but are required to recover half the cost from students' pockets, offering grants to those who cannot meet the full costs. The process of assessing students' capacity to pay is always arbitrary and sometimes invidious.

So why not hand over the state subsidy to the students, asking them to administer the communal facilities and to collect part-payment from those they consider can contribute without hardship? The students apparently calculate that they will do the job more efficiently, and fairly, than the university or the state. Rector Roman, taking the new promise of autonomy seriously, is prepared to give the scheme a try.

An even more daring scheme (still only a proposal) is that students failing to meet what is required of them in, say, five years, will be required to pay for the cost of tuition (at present free) in the extra years they spend as students. Considerable proportions at present take six or even seven years to complete their five-year course.

Hitherto, Roman says, the system has been too rigid for comfort or the educational interests of the students at the Polytechnic University. Now there will be an opportunity to concentrate on what the university does best. The university also welcomes the promise of greater flexibility in the pattern of the curriculum, previously determined in scrupulous detail by bureaucrats at the education

BIOCHEMISTRY INSTITUTE

Short commons for budding researchers

Warsaw

THE near-success of the Nazis' ambition to destroy Warsaw means that most buildings have a grey Stalinist stamp, as if the concrete had been mixed with dirty sand or even power-station waste. The Institute of Biochemistry and Biophysics, one of the Polish Academy's best institutes, also has a director's ante-room in the same style, with glass-fronted bookcases and lace curtains on the uncleaned windows.

But the comparison stops at that point. Polish institutes seem to have avoided the endemic gigantism further to the east. The institute has 130 scientists and 75 graduate students, recruited not merely from the University of Warsaw but from all over Poland. Another departure from the usual East European pattern is that the training of specialists for other laboratories and industry is a prime objective.

The research programme covers a general range of topics in molecular biology, from protein folding to the occurrence and function of D-amino acids in organisms (such as D-histidine in *Salmonella typhimurum*). But director Kazimierz Kleczkowski, himself a plant biochemist who learned much of his trade with James Bonner at the California Institute of Technology, is also proud that his institute has developed a technique based on DNA probes for screening potatoes for infection by RNA viruses.

Poland, he explains, is the "second potato power" in the world, with the result that certificates of freedom from infection are a valuable adjunct of the export trade. Perhaps more important, the laboratory has now also developed a technique for regenerating potato plants from callus tissue, with the result that whole plants can be set growing within 6 weeks. The objective is to breed potatoes resistant to infection by viruses and fungi.

In 1988, institute staff-members published 58 full papers, only 6 of them in Polish. The institute also functions as the coordinator of Poland's national pro-

gramme called the Molecular Basis of Biotechnology, which is organized by the Academy of Sciences and financed by the government's Office of Technical Progress.

In that role, the institute is among other things a source of radioactive chemicals for the 60 or so research groups scattered throughout the country. It reckons to save up to \$200,000 a year in hard currency by buying radioactive orthophosphate from suppliers in the West, converting that into the phosphorus compounds researchers need.

But even institutes whose reputation is unchallenged do not find the going easy. The institute was moved into its present quarters in 1961 for what was meant to be five years. Now the Academy is building space on another site to house up to 30 researchers, but in the present "deep economic crisis", nobody knows when the construction of other modules will be sanctioned.

Meanwhile, there are persistent fears that people will quickly be lost to the system. The institute follows the standard practice of sending its young people abroad for 1 to 3 years soon after they graduate as PhDs, and is reconciled to losing about two-thirds of those who thus go abroad. Since 1981, 22 young people from the Institute of Biochemistry and Biophysics have failed to return.

The changed political system would help were it not that salaries are so poor; newly appointed scientists (with a PhD) earn 450,000 zloty a month, senior people have basic salaries of 840,000 zloty, roughly the equivalent of \$85 a month. While directors have a certain discretion in deciding how much people are paid, there is a system by which the Academy claws back from institutes five times as many zloty as the amount by which the notional salary bill is exceeded. Plainly, there is not much scope for offsetting at the local level the consequences of Poland's dash for convertibility, which will soon bring Western prices to Polish shops.

J.M.

ministry.

There is also a plan to promote the mobility of students between universities by means of a system of credits, while the standard pattern of the five-year degree course to which Polish universities have been traditionally wedded will be loosened by the new law on degrees. There will be a four-year course leading to a bachelor's degree alongside the traditional five-year course that wins an engineering qualification.

But the details of the new pattern remain to be decided. Roman says "we are going to join Europe now", which is why the university is closely following developments in the West, with the objective of matching its own pattern with that of institutions further to the West.

That will not be easy. Despite its high reputation, the polytechnic university has had a recruitment problem. The student body has declined from 20,000 to about 13,000 now. (The result is a generous staff: student ratio of 1:5.) But Roman is looking for a 15 per cent increase in the year ahead, as well as an increase of the

numbers of students from elsewhere in Europe.

There are also problems in research. Polish universities have been luckier than those elsewhere in Europe in retaining research interests, but the shortage of equipment — especially by comparison with Western Europe — is now crippling, even inhibiting of researchers' ambitions. For the short term, Roman pins his faith on collaborative agreements with universities in the West. He is especially pleased with a recent understanding with the Fachhochschule at Cologne, under the terms of which the German university will be sending doctoral students to Warsaw.

In the present climate, the university is optimistic, the erosion of academics' salaries by inflation notwithstanding. The political transformation of Poland will not make waves comparable with those of the university's chequered past. Originally founded in 1826, the polytechnic was closed for 60 years until reopened as a Russian institution in 1898 — and solemnly boycotted by Poles until the end of Russian domination in 1915. **J.M.**

Waiting for the dust to settle

Warsaw

THE Polish Academy of Sciences, abbreviated PAN (where the 'N' means *nauki*, the Slav word for 'science'), has a new president, historian Alexander Gieysztor, who is as often to be found at the splendidly refurbished Royal Castle in the old city as at PAN's headquarters in the downtown Palace of Culture and Science.

Poland also has a kind of alternative to the official Academy, a group of 150 people organized by Professor Andrzej Ziabicki, from PAN's Institute of Fundamental Problems in Technology, which reckons to keep a watchful and sceptical eye on the process of reform. The group has been arguing that the true Academy should separate its three distinct functions as academy proper, as adviser to government and as manager of in-house research.

Gieysztor's position is straightforward — the Academy needs to be autonomous. (Here, as elsewhere, the government has held senior appointments in its gift.) Part of his objective is to give Academy institutes shop-floor democracy and to remove their control from the government-appointed secretary-general.

He sees many changes in the months ahead. Two institutes (for Socialist Countries and for Political Science) have already been abolished. Maybe there is now a need for an Institute of European Studies some institutes, on the other hand, may have to merge with university departments. The PAN Institute of Lexicography might be such a case.

But Gieysztor does not wish to see PAN lose its responsibility for research. (He says that institute directors were asked, on 17 March, whether they would favour such a change, but that they elected to stay within the academy.) In general, he is anxious that the academy's institutes should have legal independence, and that it should be possible for many of them to earn funds from other sources than the general subvention from the academy.

Gieysztor is less cheerful about Poland's future in research than many other people. Over the years, he says, the country has lost "many thousands" of talented people. And more recently, the scientific enterprise has suffered from having had an "anti-intellectual government". There are serious problems in the shortage of funds, the lack of equipment and periodicals and the poor development of computer services in Poland.

More than that, there is the difficulty that forty years of the previous regime have conditioned people to the "omnipresence of the state in private as well as public life". How, in those circumstances, will it be possible to create a market economy quickly?

John Maddox

ORGANIZATION

A new structure for science

Warsaw

THAT there should be a new way of organizing support for science in Poland is generally accepted, but it is still far from clear what pattern will emerge. The government is hoping that the new legislation will pass through the *Sjem* (parliament) in June or July, in time for the elected ingredients of the new machinery to be installed by October.

The common ingredient of most plans is a scheme by which a high-level committee of perhaps 40 people, half of them elected by the scientific community in a manner not yet specified, will advise the government at the level of the prime minister. One of the crucial elements of the arrangement will be a device for awarding research grants competitively, to groups in research institutes and universities alike.

According to a spokesman of the State Committee on Science and Technology, it has also been agreed that the device by which a large part of Poland's research and development has been financed by a 1.5 per cent levy on the turnover of Polish industrial enterprises will be abandoned under the new law.

In one sense, the change is merely a formality: so long as state ownership was the common rule, the levy was merely a means of shifting funds from one state pocket to another. In the market economy now being planned, direct government spending on research will be the only means of ensuring that research and development continues, but continuation

will depend crucially on what the government can afford.

There may be hard times ahead. In the past few years, the government reckons that its spending on research and development has exceeded 3 per cent of national income, although the figures are not directly comparable with those current in the West because of the different way in which national accounts are compiled. (The informal private sector is left out of the denominator.) But there are reckoned to be more than 110,000 people working in research and development (including university teachers), of whom only 10 per cent are employed by the Polish Academy of Sciences. The total includes 36,000 graduates, of whom 7,000 hold doctoral degrees.

In most visions of the immediate future, the research enterprise will be under severe pressure. Quite apart from the poverty of scientists' salaries, and the physical shortage of literature and equipment, there will be social pressures on the government's budget that it will not be possible to resist. One of the consequences of economic reconstruction, for example, must be dreaded unemployment, which can only be made palatable by some system of social security that does not yet exist.

That is one reason why those now pinning their hopes for Polish science on a system of competitive grants may not be nearly as cheerful a few months from now, when it may turn out that there is very little money for which to compete. **J.M.**

Rich uncle or Big Brother?

Berlin, Halle, Leipzig

THE mood of resignation and apathy gripping East Germany deepened in the week following the country's first free elections on 18 March. The election result, which gave a surprisingly large majority to the conservative parties promising rapid unification with West Germany, was seen by some researchers to reflect a lack of courage on the part of the population — "looking to the rich uncle in the West" for help, as biologist and New Forum politician Jens Reich puts it.

Well before the election, many researchers who were initially involved in the process of political change in their institutes and in society had retreated to their laboratories. "I was dancing at too many weddings at one time," says Dietrich Koch, an artificial intelligence specialist at the Central Institute for Cybernetics of the Academy of Sciences,

EAST GERMANY

Population: 16.6 million
(West: 61 million)
Area: 108,333 sq. km
(West: 248,706 sq. km)
Per capita GNP: \$10,400
(West: \$12,080)

Higher education: Seven universities and 45 other institutes. Total no. of students: 131,560 (West: 61 universities and 49 other institutes; 1.3 million students).



who nevertheless has remained active with a political party in his off hours.

At the grass roots level of research institute employees, the sense of involvement has ebbed just as dramatically. Martin Wiedmann of the Academy's Central Institute for Molecular Biology (ZIMB in German) said that in October 1989, a petition issued by the labour union stating that "we too are responsible" for the situation in East Germany was signed by 95 per cent of the employees at the institute. Now, Wiedmann estimates, just 40 per cent of the employees would sign, and in society at large, the fraction would be even lower.

Any researchers hoping for an easy life in a new unified Germany may be headed for disappointment, however. At least in the early years, unemployment is likely to rise in the new climate of competition. "My group has not made a single move to see what grants are available in the West," said one researcher. "They seem to think that money will fall from the sky."

Meanwhile, the Academy of Sciences, which supports much of the basic research in East Germany at its institutes, has announced that the budget for salaries for 1991, let alone research costs, cannot be guaranteed.

The director of ZIMB, Günter Pasternak, took a bold step and visited top sci-

ence officials in West Germany to see if the campus at Berlin-Buch, where ZIMB and two other Academy institutes are located, might be swallowed whole by West Germany and classified as a "Large Research Establishment" (GFE in German) similar to the German Cancer Research Centre in Heidelberg.

"In addition to our long tradition, we've earned the right, by doing good biomedical research, to be a GFE," says Pasternak.

Buch used to be the site of several institutes of the Kaiser Wilhelm Society, the forerunner of the Max Planck Society. "We would also like to ensure the jobs of our employees," Pasternak is virtually alone in his efforts to find a way to adapt existing East German institutions into West German structures. He has even been criticized by other directors for trying to bring his whole institute under cover when possibly only a fraction of the research will be deemed worthy of support.

Says Klaus Müntz of the Academy Institute for Plant Biochemistry in Halle, "This is not a solid road into the future." Pasternak does admit that some jobs at ZIMB will have to be eliminated in the interest of "raising the quality." But how many? Benno Parthier, the president-elect of the Akademie Leopoldina in Halle and himself a researcher in the plant biochemistry institute, takes the hard-nosed view that the number of groups that survive after unification will depend on the amount of time devoted to the transition. "Only those who can learn to be creative" in attracting money will survive, he says.

For the time being, the bulk of researchers in East Germany seem to be waiting for the best offer before deciding if they should stay in their institutes or seek employment elsewhere. The 'brain drain' that was dreaded by research policy-makers in West Germany seems so far not to have materialized. Word has trickled back, along with disgruntled researchers, that jobs are not so easy to find in the West, especially in competitive fields like biology. Even the information scientists in the Academy's cybernetics institute have for the most part stayed



Benno Parthier, soon to be at the Akademie Leopoldina, with Trabant.

home and tried to win Western contracts that they can fulfil from East Germany, says Koch.

Koch regrets that important elements of "East German culture", such as union participation in the running of institutes and generous social benefits, have not become more firmly established before West Germany swoops down and absorbs East German science. "I see little chance for maintaining any of this after the



"The Academy must be abolished", says Nover (left). "Reunification is just a word", says Rapoport.

annexation (*Anschluss*)," says Stefan Siegel of the Central Institute for Organic Chemistry of the Academy.

Worse yet, there has been little chance to help those dispossessed by the Communist system — those denied the chance to travel or even to publish for political or other reasons.

Jens Reich of ZIMB sees the fortunate few who gained favour with the Communists "still swimming like big fat fish on top of the soup". These few researchers, he fears, will scoop up the funds available from Western sources before the little fish have had a chance to apply.

Academy of Sciences

With 70 research institutes, 23,000 employees and a 290-year tradition to fall back on, the East German Academy of Sciences would seem to be the most stable scientific institution in the country in these times of instability. But paradoxically, it may be the institution with the most perilous hold on life.

East German universities will always be needed for the education of students — a shortage in university space in West Germany plus a backlog of willing students in East Germany will see to that. But who needs a "Central Institute for the Construction of Scientific Equipment" with 1,700 employees that in its present form does not match Western standards?

Despite the sweeping changes affecting every aspect of the East German economy and research scene, the Academy remains firmly in the grasp of its centralized leadership. Recreated in the Soviet image just after the Second World War, the former Prussian Academy of Sciences (founded by Leibniz in 1700) may eventually collapse under the weight of centralized research planning.

But while the academy leadership sits in stony silence in Berlin as events flow past, researchers like Rapoport and Pasternak are seeking to find their fortune in the West. DFG President Hubert Markl was bombarded with questions and appeals

Not a witch-hunt?

Halle

PRESIDENT Heinz Bethge of the Akademie Leopoldina caused a storm of debate when he was quoted in *Nature* as saying that 60 per cent of the professors in East Germany should be "thrown out" (see *Nature* 343, 497; 1990).

But despite powerful attacks, Bethge is standing by his statements. Here is part of Bethge's eloquent response to an agitated professor who warned him that one of the worst witch-hunts in German academic history could happen if his words are taken literally.

"... I am interested in two points: First of all, the great concern for the frustrated generation, that is, for the very good colleagues who were not allowed to become anything because they did not want to sell out or simply because they were feared by those who were mediocre. A second concern is for the requirement that genuine scientific performance be recognized, a requirement that was honoured less and less. Despite difficulties, I have expressed myself on both points in our country whenever it was possible..."

...It would appear to be basically accurate to say that 80 per cent of our professors were members of the 'Communist Party', and I also think that 60 to 70 per cent owe their career to their collaboration.

If anyone should feel deeply insulted at this, I should like to ask him to recall his university days and his early years as a scientific assistant. He should ask himself what became of his very good classmates, especially those who were better than himself, or, in many cases, what did *not* become of them.

I once discussed this point with some older colleagues, and it is really terrible how many of the good names that cropped up are now top people in the West. Honesty is called for here, and I have yet to hear of one of our supercareerists who has ever confessed his errors.

One could enumerate examples where real nonentities were made directors thanks to the protection of the District Committees of the [Party] ... we should not forget this.

Just one word on your 'witch-hunt,' which ... is of course nonsense. I personally do not judge simply based on appearances but I do ask: How did he act with his staff? Did he drop a good man because he thought somewhat differently? In such a case I do not want to have anything further to do with him. A politically vulnerable colleague could sometimes show some backbone, if he had one, and let a dissident [in his group] travel to a conference [outside East Germany]; this has indeed happened and with such a person there ought to be scarcely any communication difficulties..." A.A.

from Academy researchers when he gave a speech in East Berlin on 22 March.

Markl said that Academy Secretary-General Claus Grote, a member of the old guard, attended, but he did not say a word.

As for its role as a learned society, the Academy has lost all credibility, according to Lutz Nover of the Academy's Institute for Plant Biochemistry in Halle. "It's just a pile of old-fashioned Stalinists", says Nover. "Sixty per cent of them have no connections with science. They're there purely because they've been bought." Nover, who is not a member, points out that Academy members receive a bonus of as much as 1,000 East German Mark *per month* (a physician earns M1,800 *per month*).

"[The academy] must be abolished", he concludes.

Unlike other Eastern European countries, East Germany has a second, independent Academy. Because it was one of the few institutions that remained free of Communist influence, the Akademie der Deutschen Naturforscher Leopoldina in Halle stands in especially sharp counterpoint to the East German Academy.

Purely a learned society with members from the West as well as the East, the Leopoldina has become a sturdy pillar to which East German and foreign researchers have been turning with questions and offers of help.

The East German Academy has grudgingly acknowledged the role of the Leopoldina. At a recent meeting between the Academy and the Max Planck Society in Berlin, Leopoldina president-elect Benno Parthier was invited as a "fig-leaf" (Parthier's words) to cover up for the Party-tainted past of East German Academy members present.

Universities

Researchers at the seven universities in East Germany — Berlin, Leipzig, Halle, Jena, Rostock, Greifswald and the Technical University in Dresden — are still divided very strongly between the "haves" and the "have nots." The division runs almost entirely along party lines. "Round tables" and other democratic groups are still digging up the wrongdoings of current and former university administrations, and rectors are being put to the vote in many places, but in the day-to-day operation of universities, virtually nothing seems to have changed.

There are a few exceptions: the Humboldt University of Berlin has created a new and unique chair in "Social Ecology" for social scientist Rudolf Bahro, who was

hounded out of East Germany several years ago. And the Karl Marx University of Leipzig (a name that is likely to change soon) has rehabilitated Helmut Warmbier, a former professor of Marxism-Leninism who was jailed in the 1970s and later 're-educated' as an auto mechanic.

Warmbier is now coordinating the programme of 'general studies'.

But more typical is the rumour about the Leipzig mathematician who, in a closed party meeting in the tense days of October 1989, supported a policy of "shoot if necessary" to put down the demonstrations that were shaking the city. Days later, another party member who was present at the meeting placed a notice on the institute bulletin board saying "I cannot keep silent" and reporting his colleague's statement.

Not only was there no uproar or any sort

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Looking to the West: The Brandenburg Gate, November 1989.

of trial; the colleague fought back by demanding that everyone present at the meeting publicly distance him- or herself from the accusation "or else consequences will be taken" by the Party. One by one, all those who had attended the meeting backed down from the challenge. The mathematician is still teaching.

Alfred Schellenberger, a biochemist at the University of Halle, explains that democracy is not necessarily the answer in reforming a university. Schellenberger was nominated to be dean of the natural sciences faculty, but was defeated by a former Communist Party member. "Eighty per cent of those voting were in the Party," he says. "But at least the new dean promised to step down after six months." What seems to be happening in the name of "reform" is that professors who were Party members are busy taking as much of the power for themselves as they can wrest away from the former centralized power structures.

Surprisingly, the students themselves seem to be a conservative rather than a radical force. In Leipzig, says mathematics lecturer Eberhard Zeidler, some students demonstrated in favour of the Party last autumn. Now, students' main concerns seem to be maintaining their subsidized rooms and generous stipends. Zeidler said that the new "Student Coun-

cil" that has moved into the offices of the now disbanded Communist youth organization has said that it is not interested in politics; its main concern is protecting the students' "social interests".

Leipzig economics student Sven Biebler admits that East German students have played a much less active role in political change than their counterparts in Czechoslovakia and Romania. "The university warned us not to go to the demonstrations in October," he said, and most of the students obeyed and "stayed passive."

Activists like Jens Reich who had hoped to see a kind of "revolutionary new university" emerge, perhaps modelled on East Germany's own universities in the very early days of the country, have been sorely disappointed.

An East German Foundation?

Science has yet to find its voice in the new East Germany. Aside from the paralysed leadership in the soon-to-be-reorganized Academy, there is no one who can speak for the concerns of basic research the way that a science minister or National Science Foundation chief can.

Those widely separated researchers who already feel the pressure of Western competition say as one that the situation for research funding is "precarious". Tom Rapoport of ZIMB says that no one from the Academy leadership has tried to attract hard currency for research, so he has had to try to find it himself. "So far, reunification is all words," he says, "but where money is concerned, it goes much more slowly".

Rapoport and others chafe at having to apply for grants only in conjunction with West German research groups from organizations like the Deutsche Forschungsgemeinschaft (DFG). They would like to see some kind of East German Science Foundation set up to award grants on a competitive, peer-reviewed basis. But where will the money come from? In the wake of the recent elections, it is too early for the politicians to offer an answer. As of 23 March, the East German Christian Democratic Union, the party that received the most votes, had yet to appoint a spokesperson for science.

DFG says that it cannot support East German groups directly until unification. But even then, say Rapoport and others, there is an obvious need for a buffer period of two to three years before the same conditions apply for all applicants.

In the meantime, there is still the risk that the best researchers will leave East Germany entirely. Although if selected groups were "tided over" by earmarked funds from the West, as some are suggesting, then suspicion will certainly grow among rivals that connections count more than scientific achievement — a bad precedent in a newborn democracy.

Steven Dickman

Ploughing a lonely furrow

Halle

ENSLAVED but not defeated by the scourge of Marxism-Leninism, Sanskrit scholar Johannes Mehlig of Halle has taken up the challenge, along with a small group of other academics, of restructuring the historic Martin Luther University, founded in 1694.

No stranger to oppression, Mehlig endured daily beatings as the child of a Protestant minister in antireligious Nazi Germany. He suffered again for four years as a Soviet prisoner of war, beginning when he was 16 years old. After a brief flirtation with the Communist Party in the early 1950s, Mehlig thumbed his nose at the Party, which valued loyalty more than true scholarship.

These experiences, along with his recognized accomplishments in his field, give Mehlig tremendous power in his dealings with both colleagues and adversaries. "He has a towering personality," says mathematician Heinrich Renelt of Halle.

Mehlig bears his unofficial title — the "longest-serving assistant [professor] in all of Germany" — with pride and just a trace of bitterness. Stopped early in a promising career, Mehlig was "swept into a corner" by the government, says Renelt.

It would be only a slight exaggeration to say Mehlig is East Germany's only surviving dissident. Many other intellectuals were victimized by the Party all over East Germany. But Mehlig is almost alone in his refusal to strike any compromise or to



Long-suffering Johannes Mehlig . . . Now life can go on.

flee to West Germany. Hence he is one of the few people with a clean enough record to come across as a true reformer.

The main problem in the university in Halle lies in the humanities faculties, which were twisted more brutally by Communist ideology than the natural sciences. All 36 professors and lecturers in the philosophy department of the university were Communist Party members. Professors of law and economics conformed even more to the Party line, though they are now scrambling to cleanse their titles of the modifier "Marxist-Leninist."

Mehlig believes some professors could be rehabilitated, possibly by sending them to the West for a year or two, "but that there can be no pardon" for those who were guilty of "criminal acts" such as denouncing critical students or colleagues. "A teacher must be able to stand up in front of his students and be morally credible," he says.

The "Initiative Group for Renewal of

the Martin Luther University" has come only a very short way in the monumental task at hand. For example, it recently discovered that the university had maintained 15 apartments in Halle used by the dreaded Stasi, the secret police, for spying. When confronted with the evidence, the entire Senate of the university, which advises its rector, resigned.

"It's important that Mehlig stays here and stays healthy," says Renelt, who is also a member of the initiative group. "So many of the reforms here stand and fall with him." Without Mehlig, says Renelt, the rector "would not have even spoken to us."

Mehlig's rejection of Communism in 1952 came at the height of the Stalin era, when universities were expected to produce "good comrades" rather than good researchers. In 1965, he turned down an offer to become a faculty member at the university in nearby Leipzig. "I would have had to become a Marxist-Leninist Sanskritist," explains Mehlig, "which does not and cannot exist. And I would have had to deal on an equal basis with colleagues who I knew had sold out to the Stasi." Mehlig had several more scrapes with the Communist authorities before he was finally given a lifetime hold on his lowly position in the university reform of 1968. An old Jewish professor in Halle, Heinz Mode, "saved my neck by sticking me in the closet", says Mehlig. "He brought me into the department of Oriental archaeology, where they had never had a philologist before." Were it not for Mode, Mehlig believes he would not have been able to remain at university at all.

"But when Mode tried to get me a lectureship in 1967," he says, "I was denounced to the Education Ministry, which was only too happy to turn down the application. I later discovered that one of my students was blackmailed by the Stasi into denouncing me," he adds.

A ban on his publishing was lifted in 1979 after 11 years, says Mehlig, when the regime discovered that his books on Buddhism and India could be sold in the West for hard currency. One book, *Buddhist Fairy Tales*, sold 45,000 copies.

As well as helping to reorganize the university, Mehlig, now a grandfather, is intent on achieving the status — a full professorship, with freedom to travel — that he was denied for 35 years under the Communists.

Despite all he has gone through, Mehlig insists he is not bitter. "Ask my family", he says. "They always scolded me for being so optimistic. This is partly because of Buddhism, my adopted religion. But as long as 30 years ago, I realized that as soon as the Russians discovered East Germany was a burden, they would drop it like a hot potato. And then life could go on." S.D.

CZECHOSLOVAKIA

Sharp cosmopolitans break out

Prague

CZECHOSLOVAK research seems marvelously to have survived forty-two years of the old regime, largely on the strength of people's instinct for collaborating with like-minded people elsewhere. Laboratories boast of their partnerships overseas. People individually denied the right to travel to the West have made fruitful collaborations in the East.

But the system will not be able to "come back into Europe" — the common phrase — without upheaval, which will not be easy. Many institutions, conspicuously the Academy of Sciences, are in chaos (see page opposite).

Reflecting on the past, people draw attention to the importance of the Soviet-led crackdown after the Prague spring of 1968. People then considered unreliable were dismissed by the Party and, if teachers at universities, shunted into

backwater jobs. Although external contacts have remained strong, there have been fewer of them as time has passed. Last year's revolution came only in the nick of time.

CZECHOSLOVAKIA

Population: 15.5 million
 Area: 127,903 sq. km
 Per capita GNP: \$8,700
 Higher education: Five universities — Charles Univ. in Prague; Purkyně Univ. in Brno; Comenius Univ. in Bratislava; Palacký Univ. in Olomouc; Šafárik Univ. in Košice. Twelve technical universities or institutes. Total no. of students: 135,874.



backwater jobs. Although external contacts have remained strong, there have been fewer of them as time has passed. Last year's revolution came only in the nick of time.

The system nevertheless remains productive. Dr Frantisek Vyskocil, director of the Institute of Physiology, has arranged for a comparison of the productivity of his institute in Prague with that of the comparable Maria Negrino Institute in Milan. In 1988, half as many graduate scientists at Prague produced more than half as many papers in journals covered by *Current Contents* at a total cost suggesting that \$1 (US) would be worth 2.17 crowns — an exchange rate that flatters the Czechoslovak currency by a factor of more than fifteen.

The implications of the comparison will not, of course, be misunderstood: it is not the case that discovery costs only one-fifteenth as much in Prague, but that Czechoslovak scientists know the rules and work successfully to keep them.

They have to play the game with at least one hand tied behind their backs. Here are some practical problems. If you raise 15,000 crowns to buy a student's airline ticket to New York, how do you then arrange that he can pay the taxi fare to his destination when the crown is not conver-

tible? If it takes eighteen months to win approval for an order for a radiochemical, or for a hormone preparation, how do you organize your work so as to be ready to use it when it suddenly appears in the lab?

The dearth of information is crippling. Libraries have for years been cutting their subscription lists (there are said to be two libraries in Prague that subscribe to *Nature*), but the shortage of books is in many ways more serious: you cannot politely ask for a reprint. People have heard of Bitnet and the technology of the optical disk, but they know that it will be at least a decade before it is of any use to them.

CHARLES UNIVERSITY

Teaching the young is risky

Prague

LAST November, it fell to histologist Professor Zdenek Lojda to call on the then-rector of Charles University, the oldest university in Central Europe (founded in 1348), to tell him that the faculty had lost confidence in him, and he should resign.

"I'm sorry that we can't have you any longer as our rector", Lojda remembers saying. The conversation appears to have been as courteous as the circumstances would allow. "I'm sorry that I couldn't have done more", the rector said before agreeing to step down, resuming his old post as a professor in the law faculty. Lojda, who did not himself succeed in the subsequent election, is now the pro-rector responsible for overseas relations.

He tells a vivid tale of the indignities of academic life during the past 40 years. The first shock came in 1951, when the then Communist government decreed that rectors and deans should be appointed by itself, and when organs of the Party took on the task of selecting students awarded places at universities. The following year, the then new law on the Academy of Sciences removed responsibility for research from universities.

Lojda explains that, not being a party member, he was considered "a bad influence". "You see, I was a Christian". The underlying principle of academic life seems to have been that dangerous people such as Lojda should not have contact with the impressionable young. Some found refuge in research institutes, but Lojda was forced out of the Department of Embryology and Histology, and was offered refuge by friends in the Department of Pathology, but in a lowly post with few teaching responsibilities.

Lojda says that he had been able to keep his hand in histology only with the collaboration of colleagues abroad, notably in London and Dundee. He seems remark-

ably philosophical about his painful experience, noting only with regret — not anger — that the whole of his working life has been blighted by the Communist Party and explaining that he came to his decision not to comply with full knowledge of the consequences. "That's what I call freedom", he says.

Now, the objective is to put the Charles University back on the map. Lojda has been busy signing agreements with universities throughout Western Europe — Sienna, Padua, Rome, the Sorbonne, Lille and even, soon, Birmingham. The crying need is for more opportunities to travel, but also for joint projects by which people can collaborate with groups elsewhere.

Nobody is too sure what the future holds. The new law on the universities (restoring autonomy, granting open access and regulating the granting of degrees and the like) will be operative in a few weeks. But how many students will there be when the next academic year begins? Lojda reckons that the student population, now 25,000, may even increase to 35,000, so great is the unmet demand for higher education. But there are doubts about the budget.

Meanwhile, the Charles University is making a bid to contain Eastern Europe's first institute of advanced study. An international committee whose chairman is Dr Harry Woolf, past president of the Institute of Advanced Study at Princeton, hopes to recruit up to ten distinguished people to the Centre for Theoretical Study, half of them from Czechoslovakia and the other half from abroad. Some would be tenured, others on short-term contracts. People seem reasonably confident of the local contribution to the budget (estimated at 6 million crowns a year); the problem is the \$500,000 required for partial compensation of visitors from abroad and for travelling expenses.

J.M.

Making instant error-free law

Prague

DR SCARLETT Vasilukova-Reslova is the molecular biologist plucked from the Institute of Cancer Research to be deputy president of the State Commission on Science and Technology and saddled with the task of carrying through new legislation on science and technology. With Slav candour, she says, "science is in my heart, science is my soul".

She has a problem. There has to be a whole raft of legislation in the autumn — a new law on the academy, a law to create a system by which research grants will be awarded competitively and machinery to allow the federal government's conduct of public policy on science and technology to be made rationally. There is no shortage of advice. The problem is to avoid mistakes whose effects will last indefinitely.

Vasilukova-Reslova is nevertheless cheerful about long-term prospects. She says Czechoslovakia has two important factors in its favour — the understanding that "science and medicine are an important part of our cultural life" and a reputation overseas for diligent collaboration. "We are able to make bridges."

The State Commission, modelled though it is on the Stalinist pattern, is likely to survive in some form, perhaps as a regular ministry. (At present, it reports directly to the prime minister.) The government seems to accept the need to make a bridge between research institutes and the universities, and for a system of competitive grants. Vasilukova-Reslova herself is anxious that basic research is not too rigorously separated from the government's other technical interests.

Meanwhile, she spends some of her considerable energy on first aid for the Czechoslovak enterprise. Joint ventures in technical fields are in fashion, but "I'm not sure that they know what they're talking about". She is certain of the need for management training for Czechoslovak managers.

Then there is a plight of the young long denied access to the West. Vasilukova-Reslova's short-term answer is the "Foundation of 17 November" — a scheme for raising hard currency to support overseas visits by talented young people "between 15 and 30", selected not just from universities but also from high schools and research institutes. The date is significant — that of the revolution last year.

Last weekend, she found herself blessed with a new boss. The government appointed Dr Armin Delong, director of the Institute of Scientific Instruments at Brno, as chairman of the commission. Delong is a vice-president of the Czechoslovak academy and will now be a deputy prime minister.

J.M.

In the wake of Kafka

Prague

THE man describing the state of the Czechoslovak Academy of Sciences said, "It's a bit of a Kafka situation". One is not for long allowed to forget that Franz Kafka is one of this country's literary heroes. But the description of the Academy, housed in the once elegant headquarters of a commercial bank, is accurate.

The Academy is in limbo. After November's street revolution, workers at the Academy's research institutes (there were nearly 70, including an Institute for Research in Social Consciousness and Scientific Atheism) met in December to elect an alternative academy, in the ratio of one alternative academician for every 50 institute workers. This elected body, called the "lower chamber", has in turn elected seven people as an alternative management council for the academy.

By what is improbably described as a "gentleman's agreement", the president, Dr Josef Riman, was told to go, the upper chamber (popularly known as the "House of Lords" by analogy with the British Parliament) was asked to delegate management responsibility to seven members of the old management council (called the Praesidium) and this body has agreed to consider only propositions that have first been approved by the elected seven. The effect is that of a prior veto by the elected seven. It is not surprising that little day-to-day business happens.

The need for reform of some kind has been evident for years. The constitution of the Czechoslovak academy contains hardly a pretence of autonomy. The president is appointed by the government, as are the vice-presidents and the Scientific

Secretary, whose job is the administration of the research institutes. A formal procedure for the election of new members is laid down, but only the Praesidium can make nominations. The government retained the right to dismiss members of the academy on grounds such as proving "disloyal to science, the State [or] the cause of peace and socialism".

Even the upper chamber seems to agree that the old system was corrupt. The membership accreted over the years includes many people whose chief distinction is their lack thereof, or their great age. (Only seven out of 72 academicians listed in the 1987 handbook were then younger than 60, more than half were over 70.) The now common accusation that many were Communist Party hacks seems true; the monthly salary of 2,000 *koruna* academicians are paid is not irrelevant.

One member of the elected seven-man council, Vladimir Fucik from the Institute of Molecular Genetics, complained last week of the misfortune that "the Western scientific establishment accepted these people without asking questions". The bitterness springs not just from the fear that Czechoslovak science has been misrepresented abroad, but from the knowledge that between the Prague spring of 1968 and last November, the Academy's influence was to prevent visits to the West by people whose loyalty was not unquestioned.

Before last December's gentleman's agreement, the Academy had set in train a commission to reform the membership. (The harvest so far seems to be the resignation of a single academician.) But the revolutionary council is playing no part in this process. Karel Jungwirth from the Institute of Plasma Physics explains that the revolutionary council does not wish to legitimise the old regime by collaborating in its reform.

In any case, the council says, its immediate goal is to secure the future of the academy's research institutes — and its control of them. The immediate objective is that the institutes should be managed by a standing council of three representatives of the upper house and twelve from the lower, who would usually be institute directors.

On Wednesday last week, the seven-man council was hugging itself with pleasure after being told that the necessary changes to the academy law would be approved the following day by the separate republic governments and by the Federal government on Friday. There seemed a good chance that the amendments to the law would be complete before the end of April, the deadline it had set for taking action in the institutes if its demands had not by then been met.

Abrahams/Network

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

On the streets, Prague, November 1989.

"We try to follow the law as far as we can", said one member of the council.

Further ahead lie greater upheavals, on which the revolutionary council seeks to make a mark. Vladislav Hancil of the Institute of Chemical Engineering says, "we want to stop the terrible connection between science and production". Running through this conversation, as in talks with institute directors and their colleagues, is a distinctive Czechoslovak view that basic science is basic science, and must be kept inviolate.

Hancil complains that under the old regime, "we were forced to do some applied science, but it was not really applied science, only substitution". He means that researchers were required to make for themselves and others equipment that could have been bought in the West if only there had been hard currency. He adds that Czechoslovak industry has lost the capacity for innovation — enterprises have no incentive to do new things, which cause only trouble, while the funds they are supposed to spend on research and development "get lost somewhere".

What the council most wants is a new system for supporting basic science. Everybody in Prague is toying with one version or another of such a scheme. There will be a central government fund to which research groups in institutes and universities will apply for research grants, which will be awarded competitively and on the advice of referees, including appropriate people from overseas.

In the revolutionary council's vision,

Slovakia and the rest of the country would compete for separate pots of gold. To begin with, the council imagines that a small proportion of the funds available would be spent in this way, but that the proportion would then grow. At least to begin with, general support for the institutes (salaries and heat and light) would continue as at present.

Of its nature, the revolutionary council is rich in vision, but not yet acclimatized to reality. "We want to come back to Europe", says one, making the case for increased collaboration with institutions in the West. The council also seeks to bridge the gap between universities and institutes, and to take the power of decision on research funds away from the army of bureaucrats. Institute directors must be elected, and then re-elected. And basic science must be autonomous.

But what will happen if there is not enough money to go round, or if some future government asks awkward questions about the utility of present spending patterns? Four members of the council last week found it difficult to grasp the dilemma, saying that spending on basic science amounts to only 6 or 7 per cent of what the federal government spends on science and technology as a whole and that, by implication, there should be plenty of money to meet revolutionary expectations. Even the budget cuts now decreed seem not yet to have caused the council sleepless nights. It is as if that is one of those management matters that will be left to others, or just left. **J.M.**

proteases from retroviruses, as much from an interest in their biology as in their relevance to infection by human immunodeficiency virus.

Another group, continuing the interests of M. Hasek, the distinguished immunologist who was director of the institute in its previous incarnation as that for Experimental Biology and Genetics (before Riman's time), reckons to have shown that the state of immunological tolerance of a lymphocyte is determined by the population of interleukin-2 receptors on its surface, and of their condition.

Much of the institute's success stems from its reputation, which means, Riman says, that 20 students a year apply for three positions as PhD students. Such stringent selection means that they can hardly go wrong. The standard procedure is that a person is given a spell abroad after graduation. Riman says that, of these postdoctoral exports from his laboratory, only two failed to return. The institute as a whole publishes more than 90 full-length research papers a year, more than 40 a year in international journals.

Collaboration with laboratories elsewhere is conspicuous. Riman rattles off a list of institutes, which his colleagues extend: UCLA, Harvard, Torino (Turin), Imperial Cancer Research Fund and so on. Visitors to Prague are similarly regarded as the laboratory's lifeline.

But what has the institute done for the Czechoslovak economy? Riman says he "put together the case for biotechnology" in Czechoslovakia, arguing the need for innovations in fields such as beer production and cheese making. The arguments for immobilized enzyme systems have been heeded by industry.

Even now, and for as far as can be seen, the contribution of basic science to production will nevertheless be limited by the sheer immobility of the system: researchers remain anchored to their present jobs by their dependence on 'tied' apartments. And the notion that basic science and its application are two separate activities seems generally entrenched.

One of the questions being discussed last week was whether a person who has been offered a three-year appointment in London should have his position in Prague kept open for him during his absence. If it is not, there will be no easy way back for him, but otherwise, some other young Czechoslovak scientist will be denied an opportunity.

These are small issues for a proud and now lonely man such as Riman. Despite the humiliation of having been forced out of the presidency, he is fiercely proud of having retained his colleagues' respect. "Don't ask me, ask them", he says. This time round, he seems sure to be confirmed as director by election. What happens after that is anybody's guess.

John Maddox

A question of timing

Prague

DR JOSEF Riman, director of the Institute of Molecular Genetics at Prague, will almost certainly be confirmed as director when his election takes place later this month. For the time being, he is unopposed. But as recently as last November, he was also the president of the Czechoslovak Academy of Sciences as well as a member of the Central Committee of the Communist Party. Now, he is just an institute director.

Even Riman's opponents acknowledge that what has happened to him is a personal tragedy. His zeal for the development of his institute, and for Czech science generally, is not disputed. He is also given credit for having been one of the first to have urged the previous general-secretary of the Communist Party to resign (at a closed meeting of the Central Committee). His big mistake, even his friends allow, is that he hesitated too long after 17 November.

Now, in retrospect, Riman says the old system "was too tight". Of his work as president of the Academy, he says, "It

was horribly hard work — you put in 100 per cent energy to get only 20 per cent results". Czechoslovak science would now be in even better shape if the system had been more flexible. "Science needs a microclimate" that is favourable to innovation. Riman is pleased to think that he has been able to nurture that in his own institute — and his colleagues agree.

But for the system as a whole, "it was necessary to change it. We needed to be more tolerant, more flexible". Riman says his own views were influenced by a small symposium last year at Vancouver, which he attended as a private individual, not as president of the Academy. "Science is in crisis", he says. There is a need for "a higher level of generalization". He has in mind the bringing together of information theory and systems engineering with the reductionist pursuit of molecular mechanisms.

This foreboding seems not to affect the work of his institute, which has a finger in most pies now cooking in molecular biology. One group, for example, is working on the characterization of

In the market for capitalism

Budapest & Szeged

FROM a first impression, it is hard to see why the citizens of Hungary last week threw out the Communists, giving the Hungarian Socialist Party a mere 10.89 per cent of the votes in the first free elections in Hungary since 1946. The shops appear full of goods from both East and West, the people are well dressed and there is scarcely a queue to be seen. The city, with its domes and elaborate facades and mediaeval castle on the hill above the Danube, looks and feels like a great European capital.

But these are only surface impressions. The sad truth is known to all Hungarians: the prosperity they have now was bought with borrowed money in decades of 'goulash communism' during which liberal policies and full shops kept dissent to a minimum. Hungary now owes around \$21,000 million (more per capita than any other nation in Eastern Europe, including Poland), inflation has reached the 30 per cent mark, and all foreign earnings are consumed by interest payments. Industry is badly run and outdated. Much of its output is not of high enough quality to sell to the West and goes to the Soviet Union, which cannot be expected to provide a ready market much longer. Nor can the Soviet Union be relied on to continue providing cheap oil for ever, especially now that it is feeling its own post-Chernobyl energy crunch.

Another of Hungary's problems is apparent in the streets of Budapest. Fleets of inefficient Soviet-made Lada cars and poorly-maintained trucks belch out exhaust fumes, adding to the foul air from nearby generating stations that run on brown coal. Hungary has appalling environmental problems. Air pollution is particularly severe, but there is also a giant uncompleted dam on the Danube to worry about.

The economic and environmental difficulties, and the hopes that a free market

can produce some solution to them, were the driving force behind last week's election victory for the Hungarian Democratic Forum (a loose grouping of patriotic liberals) and the Union of Free Democrats (a more vigorously free-market group dominated by intellectuals). Free expression was less of an issue. Hungarians are already free to travel, see foreign movies, buy foreign books, and talk about whatever they want. Hungary's change has been a decade in the making, a gradual liberalization led by forces within the communist party who remembered that going too fast could lead back to 1956.

A free-market economy will not provide a quick panacea, although many ordinary Hungarians somehow think it will. Things will get worse before they get better. Inefficient industries will have to close. Unemployment is already appearing and petty crime is on the rise. And in a

HUNGARY

Population: 10.6 million
Area: 93,032 sq. km
Per capita GNP: \$2,010
Higher education: Four universities — Budapest, Pécs, Szeged and Debrecen — and 14 'specialized universities'; 36 institutes. Total no. of students: 66,700.



curious symptom of the breakdown of communism, street pedlars from virtually every country in Eastern Europe roam the streets and fill the subway entrance halls, selling whatever goods happen to be cheaper in their home country. The Poles are the master traders. Every city has a 'Polish market' somewhere and when the train from Warsaw arrives it lets loose a mediaeval army of ragged men and women carrying mountains of boxes and bundles.

Scientists too are as caught up in the general confusion as everyone else. The future is uncertain and many are pessimistic about the short term. For science, the

immediate past has not been so bad.

Hungary, through its network of 52 research institutes of the Academy of Sciences, plus its other Academy networks and its universities, supports a basic research network that is the most productive in Eastern Europe in terms of citation indices. Hungary comes first in the number of its scientific exchanges both with Western and with (ex)communist countries. Eight Hungarians have won Nobel Prizes, although only Albert Szent-Gyorgyi did his research in Hungary. The question now is whether a chilly competitive wind will blow over Hungary's researchers as a free-market government demands more tangible returns on its investment in science.

Visitors from the World Bank have already commented that the scale of Hungary's Academy may be out of keeping with its economic position. The new political parties have yet to give their views. Although scientists have played an active part in political reforms, the Academies and science in general are simply not on the political agenda — there are far too many other pressing problems to think about.

Academy under attack

Istvan Lang, Secretary General of the Hungarian Academy of Sciences, introduces himself half jokingly as an "old bureaucrat", with 27 years in the business of science management. The Academy occupies a magnificent old building in the centre of the capital and Lang's large office commands a fine panorama of the Danube and the hills of Buda. Despite the tranquil view, it is here at the Academy that the pressure for change is going to be concentrated, as new economic realities arrive and scientists demand a greater control over their own affairs.

Lang plainly knows that 'bureaucrats' are a little out of fashion with the coming of the free-market economy, and that there are young people who would like to see a clean sweep of the Academy; in 1986 the average age of the Academicians, who



The Hungarian Academy of Science's headquarters are by the banks of the Danube, adjacent to the famous old Chain Bridge.

retain considerable legislative power, was 69. In the agriculture section there are some Academicians who were appointed for their support of Lysenko; others have published little, and never in foreign journals.

Among the critics of the Academy are those who suspect that it will simply try to preserve itself, rather than change with the times. These suspicions were heightened last month when the Academy attempted to draft a new law for presentation to Parliament that would have made the Academy much more autonomous from the government. The draft was thrown out after the Democratic Union of Scientific Workers, the first free trade union to be created in Hungary, complained that it simply preserved the centralized decision-making system of a communist Academy.

How far should decentralization go? Some would like to see the Academy dismembered. Its research institutes, with their 3,200 scientists, would then be independent from any central bureaucratic control, or be attached to the universities rather than to the Academy.

That is an idea that outrages many of the Academy's scientists, particularly those who witnessed earlier political changes. Lajos Alföldi, director of the Institute of Genetics at Szeged, says that it is unjust to attack the Academy so much. "The Academy was organized according to the Soviet model. As with any other Hungarian institution it was strongly related to power and party", he says. "We had two choices — confrontation or creativity. Those who were inclined to confrontation went to jail and were unable to do anything except demonstrate their integrity. If you were a pragmatist and tried to accommodate to conditions you were able to perform. This was the philosophy of the Academy, which became the most liberal institution. The system of the university was mediaeval and the Academy was European. People who are pressing for the transformation of the Academy do not think of the consequences. It can be destroyed in half a year."

The dismemberment of the Academy is not in any case at all likely, however much heat is generated in discussions of the possibility. It is more probable that the future will see a continuation of the reforms that the Academy itself began some years ago. Alongside, sadly, will almost certainly come a further reduction in government support for science.

Reform under way

Lang can take credit for many of the reforms that are already in place. The most unpopular thing he says he has done is to publish a book showing the citation ratings of all papers published by 7,000

Hungarian scientists between 1981 and 1987. As the "only complete list of Hungarian scientific professionals and data on their productivity", it has proved exceedingly controversial. A recent poll of scientists shows that 40 per cent of them still think the book should never have been published, even though its spirit is exactly that of the competitive free market.

Lang has also brought a degree of competition to the grants system. In the bad-old days of central planning, money was not given out to the institutes on a rational estimation of scientific standards or productivity. Like the heads of state



This election poster for the winning Hungarian Democratic Forum reads bluntly "Comrades — it's over". The number two vote-winner in the election, the Union of Free Democrats, went for the witty slogan "Some like it free".

enterprises, the heads of research institutes saw success not in ensuring high productivity, but in seizing as large a share as possible of available resources.

In 1986 the first changes were made when Lang set up the Hungarian Research Fund (of which he remains *ex officio* chairman — "two jobs, one salary", he jokes). Fifteen per cent (4 thousand million forints; \$61 million) of the Academy's annual budget went into the fund for its first five-year period, and was given out to individual applicants after independent review of their proposals — the first time this Western system was used in the Academy.

All agree that the fund has been a success so far as it goes. University researchers are particularly pleased as they too can apply to the fund and so at long last have a chance of wresting away a small fraction of the Academy's comparative riches.

The question now is whether the Hungarian Science Foundation could end up devouring the Academy, or tearing it into pieces. The Academy is now essentially a composite of an honour body, which also negotiates exchange agreements (like the Royal Society or the National Academy of Sciences), a funding agency (like the US National Science Foundation), and a chain of research institutes (like the Max-Planck Institutes). A possible change is for the Hungarian Science Foundation to become more autonomous from the

Academy and for all research money (rather than just a small percentage of it) to be given out by the foundation, after review, to scientists anywhere.

If a greater share of the Academy's funds go into a central research fund it can do nothing but good for Hungary's universities which have traditionally been starved of funds, as well as being more hierarchial and conservative than the Academy institutes. The universities are also small, partly because they are organized in a manner like that of the faculties of Western universities; each specializes in a particular area and is attached to the Ministry of Health (Budapest's prestigious University of Medicine, for example), Agriculture (the University at Godollo mentioned below) or Education.

The universities will now have to consider whether they should join together into bigger and more comprehensive units that are compatible with the European university system. Lang says that in principle there is no reason why the universities should be attached to ministries now — the ministries do not need them. Integrating the universities will not be easy, but Lang is sure a "step by step process will start".

The system of higher degrees may also be brought more in line with that of the West. Although there is an old qualification that is somewhat similar to the Western PhD Hungary, like other East European countries, awards a 'Candidature' at the age of 28 to 35. The title indicates a capability for sustained, independent research and is essential for promotion.

Unfortunately, a candidature is also essential to apply for postdoctoral grants in the West, even though in reality it is a considerably higher qualification. A change to the Western system would ease the frustrations of young people who want to go abroad while still under 30 — although experiences at one of Hungary's best institutes, the Biological Research Centre at Szeged, show that too many of Hungary's best may be abroad already.

Brain drain at Szeged

The last relic of the Golden Age when a powerful communist Academy could establish large central research institutes is to be found in the small university town of Szeged, close to the Yugoslav border. Here is the Biological Research Centre, with its four Institutes of Biochemistry, Biophysics, Genetics, and Plant Physiology. A fifth Institute of Enzymology remains in Budapest — it contains the renegades who steadfastly refused to move to the provinces when the centre was opened in 1973.

Two hundred researchers work — or rather should be working — at the centre.

Teething troubles for biotechnology centre

So near and yet so far? The big question for the Agricultural Biotechnology Centre, the only major research institute to have been built since the Biological Centre was completed at Szeged almost 20 years ago, is whether the new government will have the will to fill it with active researchers and sustain it during its difficult early years. Otherwise what was intended as a magnificent new show piece will instead end up as a white elephant.

The design of the new institute is superb — even in part luxurious, to the embarrassment of the researchers who are trying to explain the terrible financial difficulties of Hungary to visiting foreigners. A neat trick has been to cap the institute with a floor of greenhouses. By putting the greenhouses on top rather than in a separate building, their construction costs are cut by 40 per cent alongside a similar cut in heating costs. Blinds on top of the glass roof automatically unroll when the sunlight is too strong and roll up again in high winds or snow.

Elsewhere the \$30 million spent on the institute, including a \$6 million World Bank loan, is much in evidence in new equipment, and in facilities including the latest ultracentrifuges, a Microvax computer, and a pollen-proof greenhouse area for the strict isolation of genetically engineered plants.

The institute belongs to the Ministry of Agriculture, which has its own network of research institutes and is next door to the University of Agriculture at Godollo, a forty-minute drive northeast from Budapest, through some of Hungary's few hills. Many of the institute's staff teach at Godollo, which will also provide a useful source of young researchers.

All the directors are young returnees from top laboratories in Europe and the United States and there is no shortage of good projects to be done. The centre's general director, Ervin Balazs, already has a persuasive list of ideas that will, in the long run, enormously benefit Hungarian agriculture. The trouble is keeping up the level of support needed for 4 or 5 years: "with that support we can publish good results in good journals, attract Western interest". At that stage the institute can hope to win both research contracts and more importantly, long-term support from industry. Until then, the institute must rely on the "shaky hand of government support" as Balazs puts it. The ministries of finance and agriculture are already keen that foreign investors be found. But Balazs says it is unreasonable to expect support from the West until good papers are published. "It is all right to have the facility and equipment", Balazs says, "but they have to know if the driver can drive."

The chief worry now is the brain drain that is accompanying Hungary's liberalization. Hungary's citizens are completely free to travel where they like for as long as they like. Pal Venetianer, director of the Institute of Biochemistry, explains that 25 per cent of his institute's staff are currently working in foreign laboratories.

When the institute was set up, a \$12 million grant from the United Nations Development Project ensured it could be equipped at the European level. In those days, "working here and at NIH did not feel like two different worlds", says Venetianer. But since then the level of funding has slumped, not through any change in policy but because of the deterioration of Hungary's economic situation. The result is a rush to work abroad.

A trip abroad is often essential for more than just scientific advancement. The salaries of scientists in Hungary are so low that without earning some hard currency there is rather little chance of owning a



small car, or even buying some decent furniture. At 30 years of age, few basic researchers will earn more than 8,000 forints (\$123) a month, 20 per cent less than an ordinary industrial worker. As rents are often around 2,000 forints for those lucky enough to find a place to live (and much higher in the small private sector), virtually all scientists have some kind of second job — often teaching — or have to earn money through contract research. The second job has become less profitable since Hungary took its first step towards capitalism by introducing an income tax.

When these difficulties are coupled with the many inefficiencies of daily life — the time taken to complete a shopping trip or to locate a needed item is much longer than in the West — then it is no surprise that many Hungarian researchers say that they can be ten times as productive in a good Western laboratory.

Venetianer is, however, determined to

hold the line at 25 per cent abroad. "We are trying to adopt a new policy," he says. "All the scientists want to stay away for 5 to 10 years, keeping their positions here open. But we cannot tolerate people going for 3 or 4 years, then just coming back for 6 months and going again. We are trying to be a little bit cruel and force decisions, it's not very popular!" The problem is compounded by the demand for Hungarian scientists abroad. They are already very experienced by the time they get a chance for a trip to the west. "Our senior scientists are very cheap post-docs!", says Venetianer.

How to survive?

Putting aside internal reforms, the real question for Hungarian science is how it can keep going through what are sure to be several very bad years economically. Help from the West would be much appreciated, particularly help that would allow some of the excellent scientific development. But no one expects help to arrive for nothing. One big risk is that Hungary's scientists may come to be seen as a useful resource for Western industrial development projects — given the salary levels in Hungary, it would be cheap to create some of the new products of genetic engineering, for example. An unpleasant side to this possibility is that there are many experiments which could be performed in Hungary but not in the West — the release of genetically engineered organisms, for example, is not strictly regulated.

Becoming a development laboratory for foreign companies would be far from ideal, but as Miklos Benczur of the National Institute of Haematology puts it, "It would be better than having nothing. It would be very bad for basic science, but if such collaborations can get money for science, then we would have to accept them for two to three years to survive".

If Hungarian scientists have a dream, it is that they should become a normal part of Europe as quickly as possible — participating fully in CERN, EMBO and other European organizations and having access on equal terms to European research funds. But again economics stands in the way, as Hungary needs a convertible currency before this can become possible. Lang optimistically says that this could be possible within 3 years. **Alun Anderson**

THANKS

Special thanks go to Bela Lomniczi of the Veterinary Medical Research Institute and Anna Sponaas of the National Institute of Haematology for help and hospitality in Budapest, to Pal Venetianer of the Institute of Biochemistry for hospitality in Szeged and to Lajos Nyiri, Science Attache at the Hungarian Embassy in Washington for his assistance.

ROMANIA

Life after 'Professor' Elena

Bucharest & Cluj

ROMANIA is still in a state of shock three months after the bloody revolution that toppled late dictator Nicolae Ceaucescu.

Whereas in Czechoslovakia crowds gather around public television screens to laugh at videotapes of the old Communist Party bosses, "in Romania there is nothing to laugh about", says cell biologist Maya Simionescu. Romanians still grieve publicly in the main streets of Bucharest and Cluj for the fallen.

The revolution was not exactly a surprise, say students in the medical faculty in Cluj, 300 km northwest of Bucharest. Tension began to build in May, when the government announced that, after 10 years of austerity, Romania's foreign debt had finally been repaid. But no relief measures followed. More pressure built after the Communist Party Congress in November, when the government did not announce any policy changes.

Euphoria has been replaced by fear and confusion in anticipation of the elections on 20 May. Dozens of political parties

ROMANIA

Population: 22.8 million
Area: 237,500 sq. km
Per capita GNP: \$2,540
Higher education: Six universities — at Iasi, Bucharest, Cluj, Timisoara, Craiova and Brasov — and 44 other institutes.
Total no. of students: 157,174.



have registered. "All the parties agree that there will be a market economy", says Vasile Puscas, a historian at the University of Cluj, "but they don't have a clear idea of what it means. All they are interested in is power." The current government, which was put in place by the National Salvation Front after the Front seized power from Ceaucescu, is only beginning to move clearly toward the most basic goals, such as establishing self-sufficiency in food production. Deputy Prime Minister Mihai Draganescu, who is also

President of the Romanian Academy of Sciences, thinks this can be achieved in two years.

The destruction of the economy can be attributed to the policies of Ceaucescu, who Draganescu calls an "intelligent person with an evil spirit". Science suffered more than most fields because of the meddling of the dictator's wife, Elena Ceaucescu, who fancied herself a chemist, despite her reported 4th grade education.

Elena obtained all her scientific credentials, including a doctorate from the Bucharest Polytechnic and many honorary doctorates, through exerting political pressure.

No one has received an academic promotion in ten years, says Draganescu, because Elena insisted that she was the only one to have earned it. Elena even forbade using the title "professor" on television to refer to anyone but herself, explains Daniel Cocoru, a science journalist. Other academics had to be referred to as "Mr" because Elena was supposed to be the "only true scientist" in the country.

Those expecting to find tremendous technological resources stolen from the West by Ceaucescu's spies will be disappointed, says Draganescu, who has been a full professor of electrical engineering at the Bucharest Polytechnic since 1965. Romania did have some Western technology from firms like Texas Instruments, but it is not "vital", he says. Much of what Romania could not obtain directly through a licensing agreement with the French firm Thomson-CSF "diffused" into the country via East Germany.

Reviving the Academy

The Romanian Academy of Sciences, which will celebrate its 125th anniversary next year, suffered terribly under Ceaucescu. Its 51 research institutes were detached from the Academy and members elected for purely political reasons.

But instead of disbanding the Academy as a discredited institution, Draganescu and others in government are attempting to revive it. In one of its first acts, the Front set up a 'Salvation Committee' for the Academy.

The Front drafted a new set of by-laws promising autonomy to the academy and ensuring that its purpose is to carry out basic research. Ever since the research institutes were exiled from the academy, they had to do more and more applied research to pay their bills. Consequently, they did less basic research, sometimes as little as 30 per cent in a given institute. The

Life after 'death'

Bucharest

RESEARCHERS and ordinary citizens can tell tales of being hounded, spied on, blackmailed, beaten, lied to, cheated and deprived of their rights by the corrupt Communist regime of late dictator Nicolae Ceaucescu. But few can claim the dubious distinction of physical chemist Ilie Murgulescu, who in 1986 was officially declared dead for the convenience of the regime. Very much alive, Murgulescu, now 88 years old, was featured in a February article in a Bucharest newspaper, 22, which revealed this indignity.

After completing his doctorate in Cluj in 1930 and teaching in West Germany and in Timisoara, Romania, Murgulescu came to Bucharest in 1949 and was elected president of the Romanian Academy in 1963. He was elected to four other Eastern bloc academies as well as to the New York Academy of Sciences. Since his retirement, he frequently attended official functions at the embassies of the other countries.

But soon after that the invitations stopped coming. And in 1986, an official encyclopaedia, the *Mic Dictionar Eciclopedi*, listed Murgulescu as having died in 1983. "We knew about the encyclopaedia entry," says Murgulescu's wife. "And we knew it was intentional," she adds. "[The regime] did this so they could redirect those invitations to another member of the Academy."

So far, the revolution has changed little. The invitations to re-establish foreign contacts are not yet flowing. But Murgulescu is alive and kicking, awaiting the call. . . .

S.D.

transition government has already approved an increase in the administrative staff at the Academy from 7 to 90 people in order to support the institutes.

At the same time, the Front disbanded the notorious National Council for Scientific Research (NCSR). NCSR was the front organization that allowed Elena Ceaucescu to manipulate all the scientific research in Romania. "They had to approve of everything beforehand", says Petru Filip of the chemistry institute ICECHIM. "There were so many forms", he recalls. "They must have burnt them to heat their offices." But Draganescu will not settle just for a restoration of existing institutes. He would also like to found new institutes, especially in the biological and medical sciences, and to "attract Romanian researchers back" who left the country for political or professional reasons.

Draganescu admits that it will not be easy to finance such a return to excellence. He would like to supplement the budget with income from up to ten "advanced study groups" working on specific problems like molecular engineering, materials

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

The Communist symbol used to adorn the flag. Celebrations in Bucharest, December '89.

Have institute — will travel

Bucharest

ASIDE from his mailing address, there is little to distinguish Nicolae Simionescu from other cell biologists. He publishes in international journals, edits chapters of books, attends conferences and spends as much time as he can in the laboratory.

What is unique about Simionescu is that he does all this under surreal conditions in his native Bucharest, where he is director of the Institute for Cellular Biology and Pathology (ICBP). The institute specializes in the cell biology of the cardiovascular system.

ICBP appears to be the only institute in Romania that has been able to keep up with Western labs. This is no slight to other researchers in Romania, who can be justifiably proud of what they have achieved, sometimes using considerable ingenuity to make up for a lack of resources. But in most cases, working with equipment 20 years old or more and in isolation from the rest of the world has taken a severe toll.

Simionescu, 64 years old and a physician by training, is the first to admit that setting up the institute would not have been possible had he not first left Romania to work in the United States for ten years.

Simionescu began to negotiate with the Romanian government in 1972 about opening an institute. He and his wife, Maya, were professors at the Rockefeller University in New York and did not have to go back to Romania. "My American friends called me a superstitious patriot" for pursuing the "crazy idea" of an institute in Bucharest, he says. "But I persevered." The Simionescus designed ICBP

themselves — "every mistake you see is ours," they say. They returned annually to Bucharest to supervise construction until ICBP was finally completed in 1979.

Negotiating from strength, Simionescu insisted that he and Maya be allowed to keep their faculty positions at Yale, where they had moved in 1973. And they insisted that they be allowed to "hand-pick" the institute staff of 140 and have their top people trained in the United States.

Maintaining Western contacts turned out to be essential when, in 1980, government support in the form of hard currency "dried up" and the Simionescus were on their own in purchasing books, journals and laboratory supplies. Since then, a



Simionescu: has helped the Securitate with their enquiries.

renewable grant from the US National Institutes of Health (NIH) has kept ICBP alive. The grant is the only one NIH gives to Eastern Europe and one of only 7 (out of 5,000) given to non-US groups.

"Our library became utterly dependent on donations from people we knew in the States," recalls Maya Simionescu. Nevertheless, the library became a "magnet" to

science or tunnelling microscopy. Such groups might succeed in making valuable innovations that would bring hard currency to the Academy.

The institutes themselves will also be encouraged to earn hard currency as a way of supporting their research — Draganescu said the government will take an overhead of just 30 per cent.

Previously, all such earnings were sent directly to the government.

It will be an equally difficult task to restore credibility to the academy as a learned society. Former high officials, like Ceaucescu crony Manea Manescu, currently in prison, were dismissed from the Academy without formal proceedings. But with other members, that is not even desirable, as in the case of some nonagenarian members whom no one wants to insult, says Draganescu. Ultimately, Draganescu expects the Academy, like the universities, to be cleansed "organically" of hangers-on from the Ceaucescu regime.

The Academy's problems are a microcosm of those found in society in general,

comments Nicolae Simionescu, director of the Institute for Cell Biology and Pathology in Bucharest. "Except for a very thin slice of Communist bosses, most people who joined the Party — nearly 4 million people — were just opportunists.

"They took a few privileges without hurting anybody. It would create chaos if you tried to dismiss them all." But

Paul Lowe/Network

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

The scars of revolution are far more marked in Romania than elsewhere: Street fighting, Christmas '89.

other researchers in Bucharest who could not read the literature anywhere else.

Life in Bucharest has not been easy. In addition to living with "paranoid" restrictions like only using a single light bulb in a room, Simionescu has had occasional confrontations with the Communist authorities. "Once, a man from the government came and asked us if we knew about membranes," says Simionescu. "He wanted us to produce some kind of filters for industry. We had to explain that our area is cell membranes."

The Simionescus have had to answer to the authorities in both Romania and the United States because of their frequent travel. "Both the Securitate [Romanian secret police] and the FBI thought we were selling secrets," says Simionescu.

But aside from these conflicts — and the occasional accusation of "growing up a special breed of apolitical investigators," the government "did not dare to bother us much," says Simionescu.

"Of course, we provided them every year with a propaganda coup — we kept returning from the States" to Communist Romania, he says. The revolution came none too soon for Simionescu, who says that the political situation had deteriorated dramatically in the last five years.

Since then, nothing and everything has changed. Simionescu does not anticipate receiving any new money from the government beyond what ICBP already gets, which poses problems for replacing equipment long overdue for overhaul. Nevertheless, he is "very optimistic" about the future of the institute and Romanian science in general. Says Simionescu, "At least now we can turn the lights on." S.D.

Simionescu is still uncomfortable with joining the Academy.

"It's a mixture of *Nomenklatura* [the privileged Communists from the old regime] and newcomers", he says. "They say they will slowly eliminate the *Nomenklatura*, but I am not so sure."

Universities

The one resource that the Ceaucescu regime could not destroy in Romania is talented students. Students interviewed in Cluj give the impression of being bright, hard-working and articulate, even in English. Nicolae Simionescu says, "I have taught 1,700 students at Yale and they are no brighter than the students I see here."

Since the students were the motor of the revolution the government has responded quickly to their demands. It has released students from attending lectures and seminars; reduced the sometimes absurd teaching load on the faculty; and proposed a plan to allow universities to keep 60 per cent of the foreign currency earned from the tuition of foreign students.

But many problems remain. Though

UNIVERSITIES

Parlez vous Hongrois?

Cluj

FOR the idealistic students who helped bring about the seemingly impossible revolution in Romania in December of 1989, solving the ancient problem of ethnic rivalry in Transylvania might have seemed like an easy encore.

But for the students in Cluj, a university town in northwest Romania in the heart of Transylvania, the news on 21 March of ethnic violence in Tirgu Mures, 75 kilometres to the east, dealt a dispiriting blow. The violent clashes, in which eight people died and three hundred were injured, rocked all of Romania, which is still in a national state of mourning for those who died in the December revolution.

Ethnic conflicts seem certain to present one of the largest problems faced by the new government when it takes office later this spring.

Nowhere have the tremors been felt more strongly than in Cluj, which lies on a straight line between Tirgu Mures and Budapest, closer to the Hungarian capital than to Bucharest.

The traveller's first impression of Cluj, sheep grazing next to the single runway at the airport, belies the city's economic and cultural importance. With 500,000 inhabitants, Cluj is Romania's second largest city. Ethnic Hungarians comprise roughly 30 per cent of the population, most of which is housed in the huge ugly blocks of flats characteristic of the Ceaucescu era.

One of the demands that triggered the violence was for the re-establishment of an autonomous Hungarian university in Cluj. The university has always been a focal point for dissension in Transylvania, which has changed hands between Romania and Hungary four times since 1867.

The old Hungarian university became a Romanian one in 1920, when Transylvania was given to the Romanians as part of the peace of Trianon. But the Romanian faculty was forced to flee to Sibiu in 1940 when the Axis powers gave the area back to Hungary. The university-in-exile returned after the war, and the Romanian and Hungarian universities existed side-by-side until 1959. Then they were merged into a predominantly Romanian entity with a small percentage of Hungarian faculty.

Because of the oppression and terror practised by Ceaucescu against the entire population, overt ethnic battles were reduced as all groups struggled just to survive. But now Ceaucescu is gone, says Cluj history student Bresan Ligor, a Romanian, "the feeling of commonality is less". The Romanian revolution, says medical school professor Mircea Cucuianu, had the effect of "taking the top off a

pressure cooker." Now all of the hurts suffered by both sides in the past hundred years are free to burst out.

Hungarians complain of enforced Romanization and the inherent loss of their culture under Ceaucescu. They claim that the Ceaucescu regime discriminated against them in job placement, which was all handled by the state. And they fear the trend towards nationalism that some political parties are cultivating. In a nation with 20 million Romanians and just 2 million Hungarians, they are afraid that too much democracy is not necessarily a good thing.

Having a university is important, says philosophy student Ciprian Tripon, because without one, their language will begin to seem "unnecessary" to Hungarians. Hungarian student leader Istvan Horvath puts it more strongly: "We've made a compromise already by speaking Romanian. But it's something else to have to *think* in Romanian." Most Romanians respond to the Hungarian demands for a separate university as does medical researcher Florin Nicolescu: "Nonsense. Ultimately," says Nicolescu, "they will want their own state. [Having their own university] is where it begins." Other Romanians, like medical student Maria Hategan, say they believe the Hungarians do not really want autonomy from Romania. "They just have not said this clearly enough," she says.

The Romanians have their own memories of oppression during the Hungarian *belle-epoque* from 1867 to 1920. Immunologist Horea Rus explains that his grandfather had to 'Hungarize' the family name, a bitter memory to this day. Some Romanians practised passive resistance, Cucuianu explains, by selecting Latin names like 'Troianus' that have no Hungarian equivalents.

But after several hours of lively discussion between Hungarian and Romanian students, a limited consensus emerges. "The problem really begins when these discussions leave the university walls," says Tripon. "When the peasants start to discuss it, other tensions enter in and soon it is transformed into a problem that creates violence." After the events in Tirgu Mures, the Hungarian students' association, led by Horvath among others, has changed its plans for a second demonstration for the rights of Hungarians. Instead, he says, "We're thinking of having a demonstration against violence."

S.D.**THANKS**

Steven Dickman wishes to thank Alec Pattison and Aura Vlad at the British Council; Nicolae and Maya Simionescu; and Florin Nicolescu and Horea Rus in Cluj.

Steven Dickman

everyone says that students have been able to oust professors whom they consider compromised or incompetent, in practice this has been limited to just 2 physics professors out of 600 faculty members in Cluj.

"It is hard to determine competence objectively", says Deputy Education Minister Paul Cornea. Professors do not go quietly but instead present glowing recommendations written by colleagues, he explains. Some cases may ultimately land in court, he says, but university rectors are afraid that they will lose these cases.

Research has almost disappeared at universities, says Cornea.

Philosophy students at Cluj complained that it had been years since students had been allowed to do research after completing their studies. Nearly everyone had gone directly into teaching.

Cornea confirms that this is not an isolated case.

Therefore, Cornea disagrees with the plan to reattach the institutes to the Academy. "The Academy is not necessarily the best place for the very best [researchers]", he says. "I am afraid of too much bureaucracy and centralization." But he does not have an alternative for absorbing these institutes into universities. "No one has asked me", he says.

Cluj University rector Ion Haiduc, who was elected by secret ballot on 26 January, says that these institutes could present a financial burden on the universities if they were absorbed as they are. The institutes themselves would rather be affiliated with the Academy because of "prestige", he adds.

The Education Ministry is working now on a "framework" for coordinating research and education at universities. Though there may at first be a "struggle" between the Ministry and the Academy, Cornea is confident that they will find a way to cooperate.

The universities' immediate needs mirror those of the Academy: open access to Western books and journals, travel grants for Romanian students and researchers, guest professors from outside and more modern equipment. Nearly all of these cost money, which the government does not have, says Cornea. Even the freedom to travel is not useful if there is no money for the ticket, says Haiduc. He intends to use some of the promised tuition money on travel and the rest on Western books and other supplies.

Haiduc is cheered by the many universities that have reestablished contact with Cluj since the revolution: Dijon, Rouen, Paris, Geneva and several United States universities have all expressed an interest in bilateral contacts. "Now all we need are some contacts with Great Britain", Haiduc says.

Science in the crucible

Belgrade

SCIENCE in Yugoslavia is emerging from a period of chronic underinvestment and mismanagement. The picture is changing but there is a widely shared feeling that this is a period of transition with a great many uncertainties still to be confronted.

"The old structure is like a dissolved crystal," says Dr Vladimir Glisen, head of the new Genetic Engineering Centre in Belgrade. "It will recrystallize, but into what?"

According to Professor Branislav Jankovic, of the Institute for Immunology and Virology in Belgrade, "everything is in the crucible".

This is borne out by puzzling contrasts between some research groups who find it hard to buy journals, books and everyday research materials, and others who have the very latest equipment and say they have no complaints.

The common denominator is a chronic lack of state basic research money combined with a complete switch from federal funding to funding by the individual republics in the 1970s. On the whole, the 'have-nots' are in university faculties, where research is seen as a sideline, and mathematics receives the same level of support as biology, while the 'haves' are in research institutes, and have developed links with industry or contacts with foreign laboratories. Added to these critical factors is the ideologically promising, but practically unworkable principle of self-management applied to research.

Yugoslavia has suffered unbridled economic recession and inflation for over a decade and this has been both a cause and a consequence of the malaise in science.

During the 1970s oil crisis no steps were taken to offset potential long-term economic effects. Industry remained energy-intensive, while foreign loans produced a false sense of well-being that was to be shattered in the 1980s. Instead of investing in home-grown talent, industry bought foreign technology and used it to produce goods largely for the domestic market. This created a widening gap in the balance of payments, an industrial base that was losing touch with market forces, and research almost completely divorced from economic needs.

Reforms introduced in the late 1970s, aimed to strengthen the ties between scientific research and the needs of industry, backfired.

After years of highly centralized federal research funding, responsibility was handed to the individual republics. Within each republic, science has been organized by amorphous "self-management communities of interest" (SCI's) — essentially hierarchies of committees

representing, on the one hand, the producers of research (scientists) and, on the other hand, the 'users' (industry).

The SCI's decide which projects are funded and at what rates. But in practice, according to almost ubiquitous experience, there is very little selection and grants are said by some critics to reflect the number of staff, or personal connections, as much as scientific merit.

Meanwhile, decentralization meant that federal structures disappeared. Now there is no federal 'ministry' for science — and consequently, no global budget, although very new reforms are changing this.

At the same time the richer republics, each wanting to have the best, have duplicated facilities.

YUGOSLAVIA

Population: 23.41 million
Area: 255,804 sq. km
Per capita GNP: \$2,300
Higher education: 326 institutes of higher education.
Total no. of students: 349,654.



"Leaving the people with no state help was disastrous," says Professor Vlastimir Matejic, director of science at Belgrade's Mihajlo Pupin Institute and advisor to the 1988 Organisation for Economic Co-operation and Development (OECD) review of science policy in Yugoslavia, "this has led to a typical feudalization of the system".

While there are several first class laboratories and institutes throughout Yugoslavia, the delegation of power to the republics has created what Matejic calls "negative synergy". Now, overcoming inter-republic competition to create a sufficient 'critical mass' for effective science is, he says, "the next battle to be lost or won".

One long-term consequence of the self-

management system was that the university faculties effectively stopped carrying out research.

Teaching is handled by separate "self-management communities of interest", with their own, meagre funds — the take-home pay for a senior professor is less than \$600 per month.

Now, only about 28 per cent of research is carried out in the universities, partly because of long-term hardship, partly through inertia.

By far the greatest research potential (50 per cent) is in so-called independent institutes. In addition to four large research institutes set up shortly after the war (the Mihajlo Pupin and the Boris Kidric Institutes in Belgrade, the Ruder Boskovic Institute in Zagreb and the Jozef Stefan Institute in Ljubljana), a few highly motivated university staff created their own research institutes in order to be eligible for grants and to tap industrial development funds.

But this has often meant accepting contract work and some institutes are even involved in manufacture. So, a dynamic robotics laboratory at the Josef Stefan Institute makes and sells robots and rehabilitation aids.

Similarly, Zoran Janevski's team at the Institute for Theoretical Physics, near Belgrade, makes lasers. At the other end of the spectrum, and to add to the confusion, some enterprises, such as the major pharmaceutical "companies" ('organizations of associated labour') — Galenika, Pliva and Krka — have their own research institutes and train PhD students, while senior research staff lecture at the university faculties.

Within this system there are nevertheless several top-rate laboratories, despite a significant brain drain. Some of them even manage to carry out basic research.

How is this done? The answer, very often, is a combination of foreign connections, good commercial sense and, as Vladimir Glisen has put it, "learning how the system operates". **P.C.**

International cooperation

The self-management communities of interest (SCI's) provided only about one third of research funds during the 1970s and 1980s, with the balance coming through enterprise and research organization exchanges.

As a result, agreements between Yugoslavia and foreign countries have played a significant role. The sums involved are modest, but the number of projects supported is considerable, as Blazo Krstajic, Assistant Director of the Federal Institute for International, Scientific, Educational, Cultural and Technical Cooperation in Belgrade, explained.

The major bilateral agreement is with

the USA, and started some thirty years ago when the US ploughed non-convertible dinars from trade deals back into research exchange. Today the US is a partner in 350 projects, worth \$2 million. Although the arrangements are bilateral, says Krstajic, the flow of exchange is mostly one-way. National Science Foundation training scholarships and post-doctoral programmes also allow 'bridges' to be built with US laboratories. A more exceptional case is that of Radomir Crkvenjakov, of the new Belgrade University Genetic Engineering Centre. His novel approach to DNA sequencing earned him the first US Department of

Energy foreign award under the Human Genome programme.

According to Dr Christopher Briggs, science officer for the British Council in Belgrade, these exchanges also benefit the Western partners and can be a cheap way to tap the science potential in Yugoslavia.

Significant examples, where the British Council has given grants for travel, scholarships and workshops, are in genetic engineering applied to crops such as peaches and sunflower. The Institute of Field and Vegetable Crops at Novi Sad, in Vojvodina, has developed sunflower hybrids which are revolutionizing crop yields in the USA, Europe and developing countries. Similar results have been obtained for maize at the nearby Maize Research Institute, through an exchange deal with the Max Planck Institute.

Yugoslavia also has multilateral agreements with the United Nations Development Programme for Science (UNDP) worth \$5 million for the period 1987–1991, through which 38 technical cooperation projects are supported.

UNDP funds, together with grants from the United Nations Educational, Scientific and Cultural Organisation (UNESCO) and the US National Science Foundation, allowed an important international seismic research centre to be built in Skopje, Macedonia, after earthquakes destroyed much of the city.

There is a privileged relationship with COMECON countries, particularly the Soviet Union, although, says Krstajic, this will be reviewed shortly. Still a major trading partner, the USSR values links with Yugoslavia, with its (up until recently) unique position as a 'bridge' between East and West.

One of the most coveted prospects for Yugoslavia is to join more actively in European Commission (EC) and European Community projects. Under an EC programme to develop relations with third countries, Yugoslavia has been selected as a model. Through this and similar EEC arrangements, Yugoslavia receives grants of over 10 million ECU (1 European Currency Unit = \$6.9) and is involved with 17 COST (co-operation in science and technology) programmes. After a struggle to be admitted, Yugoslavia is a third partner in four EUREKA programmes, notably EUROMAR, dealing with pollution of the Adriatic.

The Italian connection

The proximity of Yugoslavia to Italy makes these countries natural partners in science, even if in one shared project — Adriatic pollution control — Italy supplies not only some money, but also the effluent, from the Po river. But Italy is very interested in developing connections with Yugoslavia and has even recently suggested it could play a significant role

vis-à-vis Eastern Europe as a whole. In 1988 Yugoslavia signed a memorandum of understanding with Italy to improve technological cooperation, industrial development and inter-university links. So far a \$65 million research grant over 3 years has been taken up.

Several of the leading laboratories have already exploited this connection, with 11 scientific projects underway, according to Professor Stane Pejovnik, director of the Boris Kidric Institute of Chemistry in Ljubljana.

But what is stirring most interest is the so-called 'Trieste System'. Just over the border from Slovenia (Trieste even has a Slovene-speaking minority) Italy and her Alps-Adriatic neighbours see the city as a potential middle Europe research centre.

Brain drain or transplant?

Poor conditions at home and the freedom to travel abroad have inevitably created a brain drain.

But Stane Pejovnik says he would be "worried if there was no brain drain. What is important is that contacts with expatriates are good".

Indeed, he says, they represent a sort of virtual university with potential for researcher training and joint projects. And Yugoslavs are often faithful to their country. The distinguished scientist, Miloslav Radman, now at the Jacques Monod Institute in Paris is a partner in two EC projects involving Yugoslavia.

The freedom to travel abroad can also allow chance encounters, as when Beca Zlokovic from the Belgrade University Medical Faculty met the physiologist Sir Hugh Davson on London's Underground railway. This led to a 10-year pioneering collaboration on the blood-brain barrier and the admission of Davson to the Serbian Academy of Sciences.

Science policy for the future

If science in Yugoslavia gives the impression of having developed despite lack of state support, things are beginning to change. In 1987 a new federal fund was set up for the promotion of technological development. That partners from at least two republics must be involved is an incentive to overcome the 'negative synergy' Vlastimir Matejic spoke of, even if the fund is too small. A 1987 strategy for Yugoslavia's technological development has also established priorities.

But, as Dr Zlato Koren, Deputy President of the Croatian Committee for Science says, "the priorities included everything". Croatia has established its own list, with just four priorities this year, including the principle of environmental protection, since tourism is one of the republic's major earners.

Koren believes that, if any real progress is to be made, the burdensome administration of science funding has to be

changed. The Croatian 'self-management interest group for science' has 128 delegates, half of them scientists, half 'users'. But, he says "the mechanism does not work, it is too complicated".

So, this year, republics will be trying out new 'ministries' for science, where grant-making will be based on stricter criteria of priorities, and selection will be carried out by more streamlined expert panels. Although 1990 will be a transition year, the new system is expected to encourage more research competition and therefore raise standards.

In Slovenia an ambitious programme has been running for four years to train "2,000 young researchers" to PhD level. The programme runs on a six-year cycle and has absorbed over one-third of Slovenia's \$100 million annual science budget. It accepts only the brightest students and two-thirds must take jobs with industry during and after the six-year cycle. The scheme costs industry almost nothing, since salaries during training are covered by the state. Other republics such as Serbia are looking at the idea closely.

The next hurdles

As frontiers with Eastern bloc countries open, Yugoslavia's privileged status with the West will diminish. The question now is whether 40 years of experience will give the country a new role. One of the biggest problems will be the economy, as Yugoslavia's trade is still concentrated on the domestic market where competition has been virtually absent.

Most experts agree that technology has to be stimulated, but know that Yugoslavia will have to be selective.

"New technology implies social change, too", says Stane Pejovnik. In the past, he says, the state has not invested in new technology because the people were not ready for it. But in the immediate future there is little doubt that one of the most pressing questions will be whether the six republics can put aside their national interests and, as throughout history, find unity through shared adversity.

Elections in Slovenia and Croatia this month could give pointers, but common advice is "come back in five years".

Peter Coles

THANKS

Special thanks to Dr Christopher Briggs, science officer for the British Council in Belgrade, who worked tirelessly to ensure I met the right people. Ms Tatjana Lazarevic-Pivnicki of Federal Executive Council in Belgrade kindly took on the hard task of making my programme in Serbia. Her colleagues, Jadranka Lunjevic in Zagreb and Andrea Furlan in Ljubljana enabled me to squeeze the most out of my short stay.

Thanks also to Drs Jadran Lenarcic, Milo Lipovac and Doran Ivankovic for their hospitality.

Destined for greater things?

Sofia

AROUND Lenin Square, in the centre of Sofia, are a pair of Eastern Orthodox churches, a sixteenth century mosque with a tall minaret, a magnificent Stalinist-style 'Party House' complete with spire and brightly-lit Red Star, and a luxurious five-star hotel for foreign businessmen, in which only hard currency can be used. There is a statue of Lenin too, but that part of the square is under reconstruction, and he stands inaccessible, surrounded by scaffolding.

One of the churches in Lenin Square is a survivor from the mediaeval period when Bulgaria was ever at odds with the Empire of Byzantium. But after Byzantium fell, Bulgaria went too and there followed almost five hundred years of rule by the Ottoman Empire, builders of the nearby mosque. It was not until a little over a century ago that Imperial Russia came to the aid of its fellow Slavs and "freed the nation from the Turkish yoke" as Bulgarians put it. After that came a series of divisions, wars and misalliances —

BULGARIA

Population: 8.97 million
Area: 110,994 sq. km
Per capita GNP: \$6,460*
Higher education: Three universities — Kliment Ohrid Univ. in Sofia; Kirill i Metodii Univ. in Veliko Tŭrnovo; Paisi Hilendarski Univ. in Plovdiv. Total no. of students: 25,660.
*Comparable figures: USA, \$16,710; Japan, \$14,039; West Germany, \$12,080; France, \$10,740; UK, \$8,920.



including backing for Nazi Germany — which culminated in final victory for the Communist Fatherland Front in 1944.

Change is now arriving in Bulgaria yet again, but the Red Star atop party headquarters is not going to be pulled down any time soon. Out of all the old Eastern-bloc nations, Bulgaria alone stands a chance of voting for Communism in its first free elections, set for early June. The opposition is so new and so fragmented — around a hundred parties, factions and forums have been set up since reform began — that the majority may decide it is better to vote for the devil they know than the devil you don't.

But quite the opposite may happen. "A coalition government is a possibility; we cannot rule out a crisis that would lead to a new election, or even to an extreme right-wing rabidly anti-communist government — there are so many people with grievances that it may be very easy to bring them together and blame the Communist Party for everything," says Assen Jhablenski, who is both president of the Academy of Medicine and, reluctantly, a politician involved with one of the pro-

reform factions within the Communist Party.

In any case, much has already happened in the five months since the 78-year-old Todor Zhivkov, leader of the Communist Party for 35 years, was ousted. With his downfall (he now awaits trial on corruption charges) on 10 November, everyone began to speak out, and information, until then Bulgaria's most guarded commodity, began to flow. Some ugly things have come out of hiding, including secret Stalinist purges of intellectuals in the 1950s and even the size of Bulgaria's foreign debt, now \$10,000 million.

Free expression is still quite a heady brew for Bulgarians and crowds gather every weekend at the park outside the Palace of Culture for political rallies and argument. Everyone appears ready to give a frank opinion to a foreign visitor, even though the Bulgarians' puzzling habit of shaking their heads to indicate "Yes" and nodding to indicate "No", can lead to strange misunderstandings.

Heavy handedness

Bulgaria is not poor — it is richer than its Yugoslav, Turkish and Romanian neighbours — but it shows the familiar vices of heavy-handed central planning. The city is ringed with vast estates of tower blocks, identical except for the number painted on their sides, which were built to accommodate migrants from the countryside. The apartments are cramped and thin-walled, forcing an unwanted communality with the neighbours. An extensive network of rattling trams and trolley buses carry people to work (a month's travel pass costs just \$2). All the basic goods are on sale but the choice is limited and enormous efforts are needed to find items of quality. Queues form outside stores that are expecting new stock, and crowds quickly swarm around newly arriving street peddlers in Lenin Square. A fight to the centre of one such crowd revealed a youth selling a crate full of brightly coloured bendy-plastic drinking straws. It was brisk business and although few people bought more than one (and only after the most intense scrutiny) the whole stock was gone in ten minutes.

Central planning has also brought pollution to Sofia. Just outside the capital is a vast smoke-belching steel works which blankets the capital in smog when the wind blows in the wrong direction. Light industry would have been a far better choice for Bulgaria as all the iron has to be imported. But it is too late now. Other bureaucratic blundering can be corrected more easily. Last week the Parliament decided to get rid of the 'fertility encouragement tax', which had been levied on men and women who did not have

children in an unsuccessful attempt to raise the birth rate.

Strategies for success

The same defects and restrictions that afflict society have afflicted science too. Change has already begun, although it has not reached the scientist at the laboratory bench yet. Simply put, Bulgarian science does not lack good scientists but it often lacks good, up-to-date facilities and always lacks the foreign currency necessary to obtain reagents quickly and to travel easily to conferences and laboratories abroad. The result is that Bulgarian scientists never realise their potential — their productivity is limited not by their ability but by the speed with which they can work under difficult circumstances.

"Scientists cannot expect more money for research as the government will have other urgent priorities," says Professor Rumen Tsanev, head of the Institute of Molecular Biology. He hopes the election of a pluralistic government will help win funds from the West. For the time being, Tsanev says "we are solving our problems by sending scientists abroad". Up to half of the staff may go to the United States and Western Europe for 1–3 year periods while waiting for conditions to improve.

But Tsanev's laboratory is exceptional in that it is perhaps the most international of the institutes within the Bulgarian Academy of Sciences. It is one of the few in the Academy that has a deliberate policy of not publishing results in Bulgarian journals. Instead, his small group publishes 30–40 papers in well-known international journals each year. Promotion within his group depends on an "objective evaluation" of an individual's citation index and the impact of the journals they publish in. It is he says, a *sine qua non* that everyone in his group can speak English.

He says there is always demand in the West for his students as they are prepared



Rumen Tsanev, director of the Institute of Molecular Biology, has always made a virtue out of necessity; he was one of the first to separate RNA in gels successfully. "We couldn't afford centrifuges," he says. He is still bubbling with ideas despite having spent four decades in research (including a decade when care was needed not to offend against the doctrines of Lysenko) and now enjoys arguing about mathematical models of genetic networks, among many other things.

to work very hard to make the most of a short stay. "It's very sad, because I want the people here", he says. To increase the funds for his institute he has taken the familiar step of a liaison with industry. One of his groups is preparing pure interferon and he expects new agreements to be made with foreign companies.

Other institutes have similar strategies to foster good science under difficult conditions. A second molecular biology group, at the new Institute of Genetic Engineering, has the added disadvantage of being stuck out in the countryside, at Kostinbrod, an hour's journey from Sofia. The institute belongs to the Academy of Agriculture and hides away down some tortuous corridors at the back of an institute of cereal research, past a giant chicken farm. But here there is much enthusiasm and the average age of the 26 researchers is just 29 years. The stress is more on work with eventual practical application; engineering disease and herbicide resistance, and developing molecular genetic tests for disease and breed identification, are the important themes. Most of the researchers have already worked abroad and despite their remoteness, keep up active collaborations with a dozen groups in the United States and Western Europe. The work just goes more slowly and less efficiently. "I wish I could order things over the telephone, like in the United States," one of them says.

At the Institute of Electronics, a couple of researchers have found that organizing their own conferences is a neat way around the difficulties in getting the hard currency needed to travel abroad. Their international school on quantum electronics is held at the Black Sea resort of Varna and is now in its sixth year. Scientists from all over the world attend. The institute supports a diverse range of activities to keep its science going including a fruitful exchange on surface physics with Salford University in the United Kingdom, a plan to perhaps make a little money designing satellite television receivers, and a collaboration with the Soviet Union to build a receiver for a satellite that will measure the cosmic microwave background.

Of boffins and bureaucrats

At the Institute of Geophysics, a group of seven scientists (some party members, others supporting opposition groups) gathered round a large table to drink strong black coffee and iced red vermouth, a mixture certain to stimulate conversation. Some odd points emerge about the life of a scientist in Bulgaria —

the rules on state secrets being perhaps the strangest.

Bureaucratic regulations have often placed ridiculous restrictions on what can be published, according to Angel Venedikov, whose own research is on Earth tides. Magnetic survey data, geographical coordinates, and even the location of earthquake foci have at some time been considered secret. All papers published in English, even those written with co-authors while at laboratories abroad, still have to be translated into Bulgarian and sent to the State Secret Department. A Kafka-esque touch is that the people checking the papers are often unable to understand what it is they are checking. "It is typical of a pure bureaucracy not interested in the consequences of what they do but only creating and obeying their own regulations," says Venedikov. But the system is bound to change soon, and already Venedikov has exercised his



Some of the best Stalinist architecture outside the Soviet Union is to be found in central Sofia. Here the headquarters of the Bulgarian Communist Party looks down on the buildings of the Council of Ministers and the State Council. The headquarters of the Bulgarian Academy of Sciences are just down the street, next to the Parliament building.

new freedom by writing a savage criticism of the system in the Sofia newspapers.

Equally irrational are the embargos imposed by Western countries on items such as gravity meters and computers. "When we succeed in getting the hard currency then the West has an embargo," said one frustrated researcher. The difficulty of obtaining hard currency is a favourite topic of conversation. Without it, travel, attendance at symposia, purchase of foreign journals, and publication in the many US geophysical journals that impose page charges become impossible.

Venedikov jokes, "Everyone should go to the West at least for a few weeks when they are young to see that it is really not so wonderful. The ordinary person expects it to be paradise!". His colleague M. Kovacheva, a specialist in archaeomagnetism, knows at least one of the West's good points "It takes a month to get the results here that we can get in minutes on automated equipment in the West". The institute does have some automated equipment as its ground floor houses the central node for a seismic network linked to

ground stations throughout Bulgaria. The country is seismically active and has suffered two very large earthquakes this century. Results from the network are analysed on-line by a set of rather old US computers. The institute has responsibility for providing immediate information about earthquakes to government and the emergency services.

Salaries are another topic of conversation. As elsewhere in the Eastern bloc, scientists earn less than factory workers. In Bulgaria a 30-year-old scientist may earn 350 leva (\$145) a month. So almost all are forced to take some other work, once again lowering their scientific productivity.

Changing Academies

All the institutes mentioned above are attached to one of the Academies — of Science, of Medicine or of Agriculture. As elsewhere in Eastern Europe, with the increased right to speak out, the Academies have to answer criticism that they are too unwieldy and sluggish, and too remote from the universities where the next generation of scientists will be trained. They are also too fragmented — the Academy of Science alone has around 100 institutes and laboratories. It is not unusual to find almost every floor of a building has been turned into a separate institute, surely evidence that powerful personalities have demanded their own little empires.

But change is on the way for the Academies. The president of the Academy of Sciences, mathematician Blagovest Sendov, says that there are "a lot of voices saying 'kill the Academy and attach its institutes to the university' ". But he argues vehemently that it is senseless to destroy a working structure — he says his job is to "reconstruct the Academy in such a way that it will be needed for a democratic society".

Sendov appears to have support for that job. He is quite new to the post of president and has not been a member of the Communist Party, meaning he is not answerable for past mistakes. But he does not have a lot of room for manoeuvre as almost 90 per cent of Academy expenditure is on salaries. Sendov's way out of this tight corner is the familiar one of turning to links with industry in the hope that applied research can be made to finance basic research. He intends that the many institutes that carry out applied work should become "self-financing" (some of them almost are already) so that he can concentrate government funds on a smaller number of basic research institutes.

Once that is done, it will be easier to give out research grants through competitive peer review, a system that has had a chequered history in Bulgaria's Academies. At the Academy of Medicine, which has 24 research institutes and over

ASTRONOMY is one of the few areas where Bulgaria can offer world-class facilities. The Institute of Astronomy's telescopes are at Rozhan, a four-hour drive from Sofia that leads across the central lowlands to the ancient city of Plovdiv (the site of the second largest university) and then up a winding road into the Rhodope mountains. The observatory, at 1,800 metres, looks across several ranges of mountains to the Greek border.



The two-metre reflector at Rozhan.

At Rozhan are a 2-metre reflecting telescope, and associated spectroscopes, a 70-cm diameter Schmidt telescope for larger-scale sky surveys, and a 60-cm diameter telescope for photometry. The observatory represents the "beginnings of modern astronomy in Bulgaria", according to Kiril Panov, a senior member of the observatory staff who works on flare stars. Until the observatory was completed in 1981, Bulgarian astronomers either stuck to theory or went abroad for observational work.

Around 40 researchers from the institute, plus another 15 from the University of Sofia, use the observatory. Much of the work is on variable stars; besides Panov's research on flare stars, there are groups studying rotating and pulsating stars.

Several collaborative projects are under way with the Soviet Union and other countries. With the political scene changing, Panov would like to see new collaborative projects emerge; he says that the observatory is ready to offer a warm welcome to astronomers from the West.

A.A

2,500 clinical and research staff, the old system of fixing budgets for the institutes has been used until now. With such central control, one researcher explains, money goes to the person who speaks up — planning becomes more important than realization. The result has been that it is what you promise, rather than what you deliver, that determines what you get.

In the future, Assen Jhablenski, the president of the Academy, intends to switch to a much more competitive system of peer-reviewed grants, 2–4-year contracts of employment rather than automatic tenure, and a system of rewards for those who gain external contracts for their institutes. The Academy of Science already puts half its research funds into a competitive grant system that is refereed by panels of experts. But the system does not work well for the simple reason that there is too little money to be given out.

Another source of competitive funding is the Ministry of Science and Higher Education. Despite its grand title, the Ministry is considerably poorer than the Academies. It is a new creation, having recently been upgraded from a 'Committee' of the same name, whose representatives were elected by the entire Bulgarian scientific community. Its officials are now appointed and it combines the function of a National Science Foundation, with funds of around 60 million leva (\$25 million) available this year to researchers in the universities, Academies or industry. The Ministry exists in parallel with the Academies, rather than above them, but in the future might

end up swallowing the research grants of the Academies so that there would be just one central grant-giving body.

This is not a change the Academies are likely to back. Alexander Assenov, the deputy minister at the Ministry of Science and Higher Education, says that the "whole bureaucratic machine is against a central grant agency, including the directors of institutes". Assenov says, "Scientists want money and opportunities to travel and to invite their colleagues to visit them; a central fund guarantees the independence of scientists from their administrators".

With an election in the offing it is still anybody's guess what changes in the organization of science might occur. But the betting is that the Academies are so well entrenched that they cannot be broken up, nor can their power be much diminished. It is even possible that the new Ministry might find itself a committee again later in the year.

University autonomy

The University of Sofia's vice-rector Panayot Bontchev has just returned from a tour around universities in the United States, seeing how they manage their finances and their relations with new industries — surely a sign of changing times in Bulgaria. The university is the biggest and best in the country, with some 20,000 students. Its headquarters are in a classic if somewhat gloomy building just across the street from the Parliament building.

Greater autonomy has already come to the university since liberalization began on 10 November. Thanks to a new law,

Parliament will now decide the university's budget directly, and the university will be responsible for its own decisions on examinations, student numbers, the distribution of money to faculties and so on. In the past, says Bontchev, "we could only make proposals, even everyday problems had to be decided by a central bureaucracy elsewhere". The university is already busy changing its social science and philosophy curriculum — so far restricted to a Marxist approach — as a market economy appears on the horizon.

Bontchev hopes for better cooperation between the Academies and the universities. In the past, attempts at integration have failed, mainly because integration "meant decreased funds for the research at the university and an empty promise that they had access to Academy research facilities". This time, says Bontchev, instead of trying to order integration from the top, the emphasis will be on supporting research projects organized jointly by the Academy and the universities.

Hopes for increased research funds at the universities are, however, pinned on technology transfer from the universities to new small companies, some to be set up by university faculty, as is common in the United States. Bontchev says there are "lots of good ideas and techniques" at the university that could be exploited. Finding sufficient capital may prove to be the real difficulty, and the Achilles heel of both the universities' and Academies' schemes for an income from industry.

Ecological secrets

Information about the sad state of the environment is only just beginning to be made public. There are many local problems of air and water pollution, including high levels of lead around plants at Plovdiv, and a global problem in the Black Sea where pollution is serious but mostly comes from other countries.

The Academy's response has been the creation of a new Institute of Ecology with a mandate to coordinate ecological research throughout Bulgaria and to try to influence regional development and environmental policy. The biggest problem at the moment is "underestimation of the problems", says Radi Radev of the institute; "there has been no awareness of the environmental consequences of development".

Alun Anderson

THANKS

Special thanks go to Vladimir Ossenzov, Science Attache at the Bulgarian Embassy in Washington DC, and to the Bulgarian Academy of Sciences for organizing the trip upon which this article is based. In Bulgaria the many kindnesses of Magdalena Mincheva, from the Bulgarian Academy of Sciences were greatly appreciated as were the time and trouble taken by Kiril Panov to organize an expedition to the Rozhan observatory.

Energetic ion beam analysis in the Earth sciences

J.-C. Petit, J.-C. Dran & G. Della Mea

Analytical techniques using energetic ion beams are now being used to address important problems of water-rock interaction and mantle dynamics. Their unique capability is to probe the outermost few micrometres of minerals and glasses, providing multi-element depth profiles of this very important surface layer.

IMPORTANT questions in the Earth sciences, relevant to both the crust and the mantle, involve processes that occur at interfaces. Chemical changes in the near-surface regions of minerals (to a depth of $\sim 1 \mu\text{m}$), and particularly the resulting elemental depth profiles, are keys for identifying the mechanisms involved in both solid-liquid and solid-solid interactions. To the first category belong chemical transformations occurring during water-rock interactions, such as the aqueous dissolution of minerals and glasses, the precipitation of secondary phases and the retention of species by mineral surfaces. In particular, processes such as ionic diffusion, ion exchange, permeation of neutral species, network hydrolysis, condensation and adsorption—all involved in aqueous dissolution—have been identified and extensively investigated by a few pioneering teams using ion-beam analysis.

Surface physico-chemical modifications are also important in metamorphism, in which the mineralogical makeup of a rock is transformed in response to high temperatures and/or pressures, such as those attained during mountain formation or the intrusion of hot magmas into the crust. Although the important role of fluids in metamorphic reactions is now recognized, reactions at solid-solid interfaces cannot be excluded. The crystallization of minerals from silicate melts can also be studied using elemental depth profiling, as changes in chemical composition at the interface between crystal and silicate melt reflect the chemical evolution of the magma.

The analytical tools commonly used to study the composition of geological materials, such as electron probe microanalysis (EPMA) and secondary-ion mass spectrometry (SIMS), cannot easily give such information in a quantitative and non-destructive way. Fortunately, techniques based on energetic (MeV) ion beams, mostly developed in the past decade, allow depth profiling of a wide variety of elements with the two above characteristics and, in addition, with good depth resolution. These techniques are derived directly from nuclear physics and are already applied commonly in materials science and solid-state physics.

Based on the detection and energy analysis of the interaction products of energetic ($\sim 1 \text{ MeV}$ per nucleon) ion beams, supplied by small particle accelerators, impinging on solid surfaces, these tools include those relying on resonant or non-resonant nuclear reactions, elastic scattering by target nuclei and ionization-induced X-ray emission. This last technique, called particle-induced X-ray emission (PIXE), is similar to EPMA, but extends its sensitivity to trace elements, albeit with a poorer depth resolution ($>1 \mu\text{m}$). As the application of PIXE in Earth sciences has already been reviewed (see, for example, ref. 1), this technique will not be discussed here. The techniques described here, nuclear reaction analysis (NRA), Rutherford backscattering spectrometry (RBS) and elastic recoil detection analysis (ERDA), provide depth profiles of element concentrations with excellent depth resolution (in the range 3–40 nm for common silicates).

MeV ion-beam techniques can also be used to study geological phenomena that do not involve interface processes. Such

phenomena, one example of which is convection in the mantle, involve the very slow transport of very large amounts of matter. They induce gradients of chemical composition in the Earth, which can be used to infer fundamental parameters of geo-physical significance, such as pressure/temperature conditions, or diffusion coefficients. Laboratory simulations can be performed to reproduce these chemical gradients, but only on extremely small spatial scales, given reasonable experiment durations and realistic temperatures. Several studies concerning mantle dynamics have thus benefited from precise measurements of ionic self- or hetero-diffusivities in mantle constituents.

In the following sections, we will first briefly describe the physical principles on which these techniques are based, highlighting their main advantages and limitations as compared with more classical analytical techniques. We will then review the application of these new tools during the past few years to issues relevant to both crustal and mantle phenomena. Finally, we will try to provide a few guidelines for extending their use to other important problems in the Earth sciences.

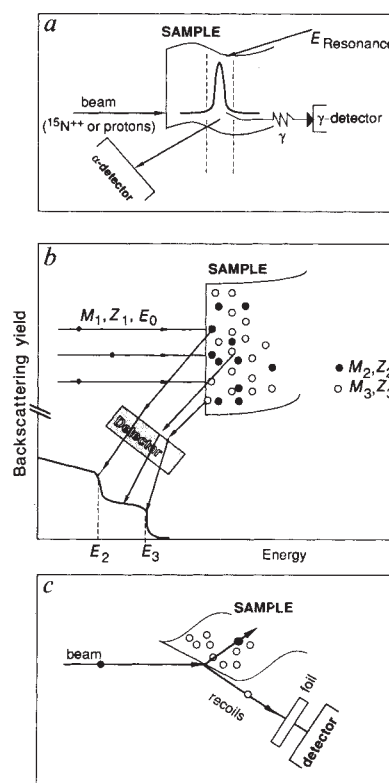


FIG. 1 Basic principles of the three ion-beam techniques (see text). a, Resonant nuclear reaction analysis; b, Rutherford backscattering spectrometry; c, Elastic recoil detection analysis.

Description of techniques

MeV ion-beam techniques have the following common features: they rely on the bombardment of a flat solid target with a beam of monoenergetic charged particles of low mass and high energy, supplied by small accelerators. Such particles interact with the nuclei of target constituent atoms and generate a variety of secondary products, ranging from charged or neutral particles (α -particles, neutrons, electrons, the scattered ions themselves) to photons (X-rays, γ -rays). The analysis is based on the detection of these interaction products; in many cases it is facilitated by the use of appropriate standards. The three techniques considered here, NRA, RBS and ERDA, are used for the analysis of, respectively, specific light elements (particularly hydrogen), intermediate and heavy elements, and isotopes of light elements (such as hydrogen and deuterium).

NRA (Fig. 1a) uses a nuclear reaction specific to an isotope of the element of interest and induced by an ion beam of appropriate species and energy². Reactions exhibiting sharp resonances in their cross-sections (directly related to the probability of interaction), are preferentially used for depth profiling (resonant nuclear reaction analysis or RNRA), because they yield a good depth resolution. (As an energetic ion penetrates a solid, it loses its energy gradually, in a predictable way. This dependence of ion energy on depth is the key to all of the techniques discussed here, and explains why a narrow width in energy is translated into good depth resolution.) For example, sodium depth profiles can be obtained by means of the $^{23}\text{Na}(p, \alpha)^{20}\text{Ne}$ nuclear reaction, which has a resonance at 592 keV, with a narrow width of about 0.6 keV, determining a depth resolution at the surface of the order of 10 nm in common silicates³. The profile is determined by measuring the α -particle yield with depth, by increasing the energy of the incident protons in steps from the resonance energy. (A step of 1 keV corresponds to a step of 16 nm in depth; see Fig. 1a). Another resonant nuclear reaction, $^1\text{H}(^{15}\text{N}, \gamma)^{12}\text{C}$, is particularly useful for hydrogen depth profiling. This has a resonance at 6.385 MeV, a width of 1.8 keV, and the detected radiation is the γ -ray. In the case of silicates, the depth resolution is 3 nm at the surface and 10 nm at a depth of 100 nm (ref. 3). In some cases, non-resonant nuclear reaction analysis can be used, mainly for measuring the total surface concentration of a particular isotope (^{12}C , ^{14}N , ^{16}O , ^{18}O , for example), but also for depth profiling when a depth resolution of a few nanometres is not needed⁴.

A detailed description of the **RBS** technique can be found in several extensive reviews (see, for example, ref. 5). RBS is based on the elastic collisions arising from Coulomb interactions between incident energetic light ions (typically helium in the range 1–4 MeV) and the nuclei of target atoms. The collision process is described by classical kinematics, with well-known probabilities of interaction (Rutherford differential cross-sections). A small fraction of the ions passes close enough to the target nuclei to undergo large-angle elastic scattering (backscattering). The backscattered fraction of the beam is detected at a fixed angle and analysed in energy by a silicon surface-barrier detector (Fig. 1b). A typical RBS spectrum of a polyatomic target exhibits successive steps: the edges correspond to specific atoms located at the surface, and the heights of the steps give their concentrations. Information about the depth distribution of the scattering atoms is provided by the energy distribution of the backscattered helium ions around each edge, resulting from the energy loss on both the inward and backward trajectories. A depth resolution of 25 nm can be achieved at present.

ERDA is an alternative to NRA for measuring the depth profiles of the concentration of light elements in solids^{6,7}. Like RBS, it relies on elastic scattering, the only difference being that the incident ions must have a greater mass than the target atoms to be profiled. The lighter atom recoils in a forward direction and, if the sample is tilted, can escape and its energy can be analysed, thus providing information on its original depth. To prevent scattered probing ions (commonly 28-MeV ^{28}Si or 2.2-

MeV ^4He) from interfering with the light recoil atoms, a thin absorber foil is placed in front of the detector. The depth resolution is determined by the energy loss of both the incident ion and the recoiling atom (Fig. 1c) and is of the order of 50 nm.

Advantages and limitations

The primary advantage of these three techniques is that they are non-destructive and quantitative. In contrast to other techniques yielding elemental profiles, such as secondary-ion mass spectrometry (SIMS), X-ray photoelectron spectrometry (XPS) and Auger spectrometry, they provide the depth distribution of analysed elements directly without requiring sputter erosion of the surface. This last procedure induces elemental redistributions, as well as removal of material. Moreover, the excitation function, which governs the production of the detected signals, is an intrinsic property of the nucleus of the analysed element and is not sensitive to chemical environment. Consequently, energetic ion-beam techniques are not limited by matrix effects as are SIMS, XPS and Auger spectrometry. Another advantage is their excellent depth resolution. Furthermore, MeV ion-beam analysis minimizes the difficulty of handling insulating materials compared with other techniques that rely on charged particles (such as Auger, SIMS and EPMA), because the current densities involved are more than one order of magnitude lower.

Energetic ion beam techniques also have more specific advantages. For instance, RNRA is an almost unique tool for hydrogen depth profiling, the only other (SIMS) often leading to questionable data. This feature is particularly important in Earth sciences, because of the ubiquitous occurrence of water-rock interactions. The specific advantages of both RBS and ERDA

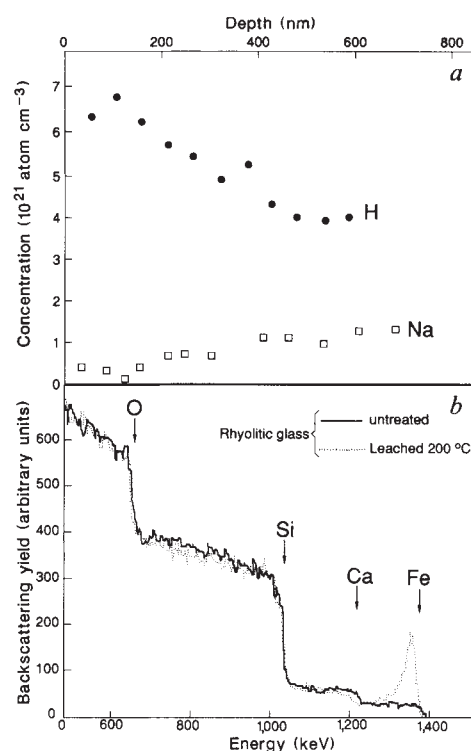


FIG. 2 Dissolution features of a rhyolitic glass (Santa Fe, New Mexico, USA) after 13.5 h in deionized water at 200 °C (ref. 14). a, H and Na depth profiles, obtained by RNRA (respectively by the $^1\text{H}(^{15}\text{N}, \gamma)^{12}\text{C}$ and $^{23}\text{Na}(p, \alpha)^{20}\text{Ne}$ reactions), extend to depths larger than 600 nm, thus exceeding the analytical capabilities. Anticorrelated shapes indicate an ion-exchange process; note that, in addition to sodium, this glass also contains 2.8 wt% K_2O which is probably involved in this process. b, The RBS spectrum, compared to that of the untreated glass, exhibits a sharp Fe peak which can be attributed to the formation of a Fe hydrosilicate, as inferred from spectrum deconvolution.

are their extremely easy implementation and the rapidity with which multi-element depth profiles can be obtained.

These techniques do, however, have several limitations: they have a relatively low sensitivity (they are generally not suitable for trace elements), compared for instance with SIMS or neutron activation analysis (NAA), and have poor lateral resolution (of the order of 0.1–1 mm²). This is due to the difficulty of obtaining small and intense beams with common accelerators. This feature greatly hampers the direct application of these techniques to the analysis of specific phases in multi-component geological samples. Until now, their use has been restricted to the characterization of a few millimetre-sized and flat samples prepared for laboratory simulations. Moreover, despite the basic non-destructive character of hydrogen depth profiling with ¹⁵N, hydrogen mobility under the beam is often observed⁸, which requires reduction of the current density to the lowest practical value (typically <100 nA cm⁻²). In addition, no information on chemical bonding can be obtained, as with XPS. Finally, the implementation of these techniques necessitates the use of nuclear facilities which, until recently, were not easily accessible to the Earth sciences community. However, small particle accelerators are used less and less by nuclear physicists and therefore are becoming more available to other scientists. An example is the development, in recent years, of accelerator mass spectrometry⁹, which is now routinely used for the high-sensitivity analysis of ¹⁰Be, ²⁶Al and ³⁶Cl, with increasing applications in Earth and planetary sciences.

Application to Earth sciences

Early applications of ion-beam techniques to geological materials involved the depth profiling of hydrogen in quartz and silica⁸, and obsidian¹⁰. These studies were not, however, aimed explicitly at contributing to Earth science issues. Instead, they were intended respectively to develop the methodology of hydrogen depth profiling on a variety of materials and to calibrate the dating method of archaeological artefacts, based on their hydration kinetics. Our own interest in this field stemmed from our previous contributions to issues of materials science such as the durability of technological silicate glasses, including nuclear-waste-bearing glasses deposited in deep geological formations^{3,11,12}.

In recent years, the unique capability of such techniques to provide the depth distribution of light elements in the outermost micrometre-thick region of geological materials has been fruitfully applied to two main research areas. First, they have allowed the direct analysis of hydrogen on reacted mineral surfaces, which is the key to understanding mechanisms of water-rock interactions in the Earth's crust. Second, the measurement of diffusion coefficients of specific elements in common silicate minerals has contributed significantly to the modelling of mantle dynamics.

Water-rock interactions. The Earth's crust results from an equilibrium between dynamic accretion phenomena which originate in the mantle (see next section), and destructive forces that consume crust, among which water-rock interactions are dominant. Moreover, important problems of economic and technological interest are directly linked to such issues. The study of water-rock interactions is of prime importance in the Earth sciences, particularly in modelling the mass balance of elements during their geochemical cycle. To achieve such a modelling, it is necessary to decipher whether, like technological silicate glasses, natural glasses and silicate minerals dissolve stoichiometrically. In the former case, the reacting surface maintains the same bulk composition throughout the dissolution process, whereas in the latter, some elements (mainly, alkalis and alkaline earths) are preferentially released in solution, and others (such as silicon, aluminium and transition elements) accumulate on the surface¹³. The resulting alteration layer can be characterized in detail by ion-beam analysis, allowing the identification of dissolution mechanisms.

For natural glasses, we showed that several mechanisms occur simultaneously during dissolution, namely ionic diffusion, ion exchange, permeation of neutral species, adsorption of dissolved species, network hydrolysis and condensation of silanol (Si—OH) groups¹⁴. The overall phenomenology of dissolution results from the competition between these mechanisms, which are in turn constrained by the glass composition, temperature, pH and the solution composition. We could then discuss in detail the stoichiometry of glass dissolution, the nature of the alteration layer formed on the surface and the correlative retention of transition and heavy elements. For example, we have shown¹⁴ that, in pure water and at moderate pH, a basaltic glass dissolves quasi-stoichiometrically, with the exception of a reaction zone a few tens of nanometres thick, detectable only with high-depth-resolution techniques. The alteration layer (palagonite) therefore probably consists of 'precipitates' (discrete secondary phases formed *in situ* without distant release in solution). Deconvolution of RBS spectra suggests that the first corrosion stage results in the precipitation of an iron hydroxide on the glass surface, a process already reported for the interaction of basaltic glass with sea water¹⁵. A detailed knowledge of the corrosion features of basaltic glass in sea water is needed for the determination of the mass balance of elements at mid-ocean ridges¹⁶.

In contrast, for rhyolitic glass of much higher silica content, we concluded that dissolution was selective, governed by the exchange of hydrogen species for alkali elements (sodium and potassium) in the glass¹⁷. We also showed that elements such as iron, titanium and uranium tend to be retained preferentially in the hydrated layer, at least as long as it remains quasi-amorphous. In this case, RBS spectra indicate the formation of a surficial hydrosilicate (Fig. 2). Moreover, we suggested that the diffusion of water molecules becomes a dominant process of glass dissolution at high temperature (>200 °C). These conclusions have implications for understanding the formation of some uranium ore deposits associated with the alteration of rhyolitic glasses¹⁸.

The main contribution of these analytical tools in problems of mineral dissolution was to show that the basic processes involved are the same as those active in glass dissolution, not-

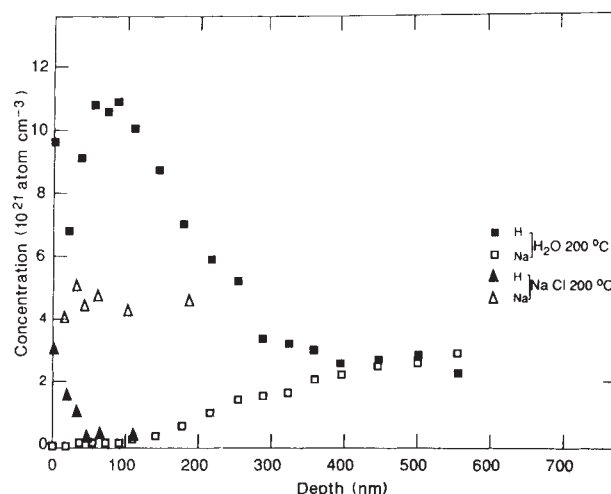


FIG. 3 Dissolution behaviour of albite after two days at 200 °C in deionized water or a 250 g l⁻¹ NaCl solution²³. Strong evidence for ion exchange is deduced from both the anticorrelation between the H and Na profiles and the marked hindrance of mineral hydration in the presence of a sodium-rich solution. Quantitative analysis of the profiles (comparison at each depth of released sodium with incorporated hydrogen) suggests that a complementary process is involved, either diffusion of water molecules if protons are exchanged with sodium ions, or recondensation of silanol groups if hydronium is considered (see text).

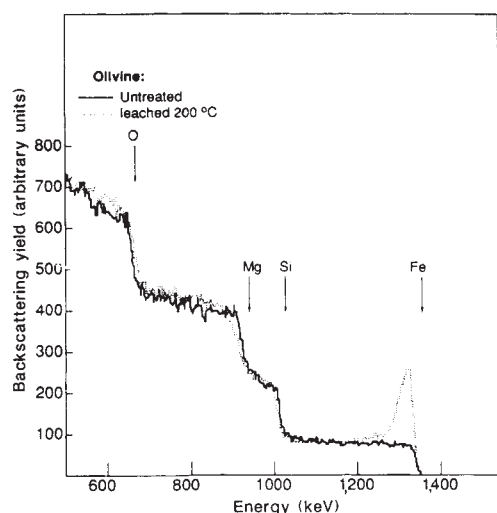


FIG. 4 RBS spectra of olivine before and after dissolution in deionized water at 200 °C for 6 d (ref. 28). The sharp Fe peak is the signature of iron hydroxide precipitation on the mineral surface, as deduced from spectrum deconvolution. Correlated H profile (not presented here) shows the absence of hydration for the underlying olivine²³.

withstanding the apparent phenomenological dissimilarities related mainly to the stoichiometry of dissolution. Previously, this issue was addressed mainly through solution analyses, which give only indirect information concerning chemical modifications at the mineral surface. A few studies relied on surface characterization by XPS^{19–21}, which unfortunately does not provide analysis of hydrogen and thus direct information about hydration processes. The first contribution to mineral dissolution with geological relevance showed, by hydrogen depth profiling with RNRA, the presence of an important surface hydration during the aqueous corrosion of diopside²². Complementary SIMS data on principal constituent atoms allowed us to ascribe this feature to stoichiometric dissolution at reactive sites, followed by permeation of molecular water in the resulting porous structure. Subsequently, however, we demonstrated²³ that crystalline silicates can behave like silicate glasses in forming, under certain conditions (high temperature and/or low pH), a hydrated layer, which results in overall non-stoichiometric dissolution (Fig. 3). The silicates that most easily form a hydrated layer are those containing exchangeable cations and a high proportion of connected silica tetrahedra.

Good examples of such silicates are the alkali feldspars, for which we have demonstrated²³ that the formation of the hydrated layer at moderate pH is due to ion exchange between the alkalis and hydrogen species. Because the ratio of hydrogen uptake to alkali release departs from 1 (in the case of H^+) or 3 (for H_3O^+), a simple ion exchange involving either one of these species could not be advocated. Two hypotheses could then be proposed: either an ion exchange with protons accompanied or followed by diffusion of water molecules²³, or an ion exchange with hydronium combined with partial condensation of the silanol groups, eliminating some water ($2 Si-OH \rightarrow Si-O-Si + H_2O$). The latter hypothesis was confirmed recently²⁴ for the dissolution of labradorite feldspar in acidic solutions (pH 2–3) by performing a hydrogen-balance calculation. In addition, this investigation proved that a marked H-D isotope exchange occurs between the silicate and the solution, associated with hydrated-layer formation. It is likely that a similar exchange occurs among oxygen isotopes for most minerals, as reported already for silicate glasses²⁵. Indeed, it has recently been demonstrated²⁶, by means of leach tests of a pre-hydrated labradorite feldspar with ^{18}O -labelled water, that this mineral also rapidly exchanges oxygen isotopes with the solution over the whole thickness of the surficial silica layer. Such a process could have important consequences for the use of isotope ratios of hydrogen and oxygen measured in rocks. In addition to the alkalis, calcium also participates in the ion-exchange process²⁴. This must be considered in the overall mass balance involved in formation of the hydrated layer.

An illustration of the role of the silicate network structure is given by results for olivine (Fig. 4), which demonstrate the absence of any hydration on this mineral, in spite of a significant concentration of exchangeable magnesium. In our judgement, this is due to its structure, formed of isolated silica tetrahedra, which does not allow the build-up of a stable residual silica network after cation release. Thus, only network silicates (three-dimensional networks) can easily generate hydrated layers, the only exception being quartz, in which no exchangeable cation is available. Our observation of a marked hydrogen penetration in reacted diopside²², which is a chain silicate, does not contradict this proposal, as such a hydrogen uptake does not correspond to an actual network hydration (as mentioned above, the SIMS data indicate stoichiometric dissolution at reactive sites followed by permeation of water into the porous structure). Our interpretation of the dependence of hydrated-layer formation on the silicate structure is confirmed by our investigation of ion bombardment effects^{27,28}. We have shown that the introduction of high densities of radiation damage in a series of different silicates transforms their specific structures into glass-like ones, which then exhibit responses similar to hydration without any structural dependence.

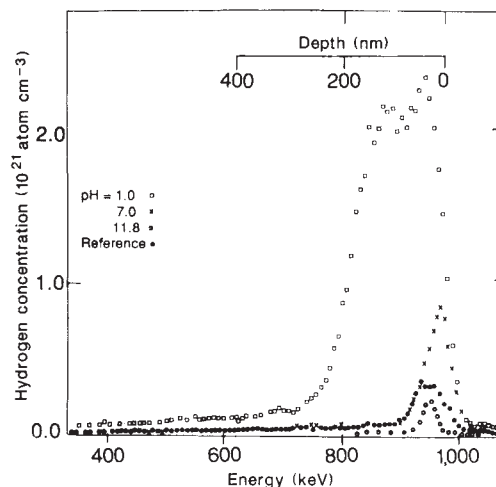


FIG. 5 ERDA hydrogen spectra of labradorite subjected to water attack at 45 °C for 264 h and at various pH values ranging from 1.7 to 11.8 (ref. 31). The amount of hydrogen and depth to which it penetrates markedly decrease with increasing pH, and become even lower than those of the reference material for a very basic solution. This last feature is probably due to an enhanced network hydrolysis, leading to congruent dissolution. The reference profile is that of untreated labradorite.

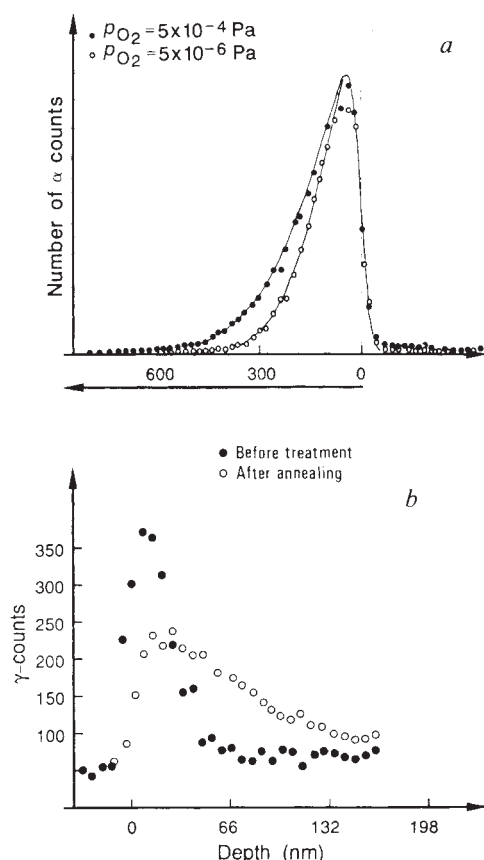


FIG. 6 Depth profiles of elements of geochemical significance supplied by RNRA with the aim of estimating diffusion coefficients in mantle constituents. *a*, Profile of ^{18}O obtained by means of the non-resonant $^{18}\text{O}(\text{p}, \alpha)^{15}\text{N}$ reaction (incident proton energy of 750 keV) in olivine put into contact, for ~ 40 h at $1,300^\circ\text{C}$, with a gas mixture of Ar and H_2 saturated at room temperature with H_2^{18}O (ref. 34). The energy spectrum of secondary α -particles is deconvolved into a depth distribution (along an inverse scale) but, owing to a lack of experimental information, the original energy scale cannot be given. *b*, Depth profile of aluminium in diopside, obtained by the $^{27}\text{Al}(\text{p}, \gamma)^{28}\text{Si}$ reaction (resonance energy of 992 keV) after coating the mineral with an Al-rich gellous film and heating at $1,180^\circ\text{C}$ under controlled low oxygen fugacity (10^{-8} Pa) for 16.8 d (ref. 36). Note that the authors did not provide absolute oxygen or aluminium concentrations. An inward diffusion of oxygen or aluminium results from an increase in p_{O_2} or a thermal treatment respectively.

The conditions favouring the build-up of the hydrated layer are those enhancing the processes of hydrogen penetration (for example, ion exchange) relative to the processes responsible for layer destruction (such as network hydrolysis). In particular, we have shown on albite²³ that a high temperature ($>200^\circ\text{C}$) leads to a relatively thick (a few hundred nanometres) hydrated layer. Even olivine, treated at a temperature of $>600^\circ\text{C}$, seems to exhibit a detectable hydrated layer, as we observed recently (work in collaboration with S. Fujimoto and B. Velde). In contrast, the decrease of the chemical potential gradient at the mineral-solution interface, for the mobile species involved in the ion-exchange process, considerably hinders hydration with respect to network destruction²³ (Fig. 3). Similarly, ERDA measurements on leached labradorite feldspar²⁹ have shown that both the thickness of this latter and its hydrogen content markedly increase with decreasing pH, down to a value of 2, whereas dissolution becomes stoichiometric at high pH values (>10), because hydrolysis of silica is then dominant (Fig. 5).

Another similarity between silicate minerals and natural glasses stems from the surface accumulation of easily hydrolysable elements (such as transition elements, lanthanides and actinides) during dissolution. For example, olivine leached at 200°C and moderate pH exhibits a marked iron accumulation (Fig. 4). Deconvolution of the RBS spectrum, combined with hydrogen profiling, allows one to infer the precipitation of an iron hydroxide. In fact, under such experimental conditions, this compound, which is highly insoluble, readily precipitates on the olivine surface. In other dissolution conditions (different temperature or solution composition, for example) other secondary compounds can form (for example, hydrosilicates). Elemental surface enrichment also occurs by sorption, when metallic elements initially in solution are trapped by mineral surfaces³⁰. These phenomena are common in the natural environment and are of prime concern when investigating such topics as the geochemical cycle of elements, the formation of ore deposits and the dispersion of pollutants in the solid Earth,

such as the radioelements contained in nuclear waste deposited in geological sites.

Mantle dynamics. More recently, ion-beam analysis has been used to address two problems involving the properties of the Earth's mantle. The first is the mechanism of creep in the mineral olivine, which comprises at least 65% by volume of the upper mantle. Creep in olivine is thought to be the main deformation mechanism in the upper mantle, and is therefore important in convection models³¹. The second problem is the mechanism by which aluminium-rich phases (plagioclase, spinel and garnet) are formed from clinopyroxenes, which constitute a few per cent of ultrabasic rocks and more than 50% by volume of pyroxenites originating in the upper mantle. These solid-state mineralogical reactions are used as geothermometers and geothermobarometers, in that the compositions of coexisting minerals can be used to infer the pressure and/or temperature of equilibration³². Such reactions, which require the diffusion of constituent atoms, occur at extremely slow rates in nature and are thus difficult to simulate in the laboratory. For example, the diffusivity of silicon in olivine is of the order of $10^{-20} \text{ m}^2 \text{ s}^{-1}$, which corresponds to a diffusion length of ~ 150 nm after one week at $1,600^\circ\text{C}$. Quantitative measurements relying on chemical gradients over such small thicknesses necessitate the use of analytical tools having extremely high depth resolution, such as the MeV ion techniques considered here.

Until recently, the nature of the species controlling the rate of the high-temperature creep of olivine in the mantle was not clearly identified, with the proposed models being based on the slowest-diffusing species (oxygen or silicon)³³. The most mobile constituents (magnesium and iron) are responsible for the mantle's ionic conductivity. A model based on the coupled diffusion of the entire olivine molecule ($2(\text{Mg}, \text{Fe})\text{-1Si-4O}$) was proposed³⁴, which relied on the measurement of the diffusion coefficients of oxygen and silicon by the reactions $^{30}\text{Si}(\text{p}, \gamma)^{31}\text{P}$ and $^{18}\text{O}(\text{p}, \alpha)^{15}\text{N}$, respectively^{4,34}. In the temperature range $1,000\text{--}1,600^\circ\text{C}$, it was shown that the order of the diffusion

coefficients of the species was: $D_{Fe}, D_{Mg} \gg D_{O} \gg D_{Si}$ (see Fig. 6 for oxygen diffusion), and that their variations with partial pressure of oxygen (p_{O_2}) obey different laws. It was thus concluded that the creep of olivine is controlled not by a single species but by mixed diffusion of all constituents, associated with an internal electric field coupling their fluxes. In addition, the variations of these coefficients with p_{O_2} revealed the basic processes involved: diffusion by means of vacancies for magnesium and iron and of interstitials for oxygen.

The second application derives from the strong pressure and temperature dependence of aluminium solubility in pyroxene, which can be used to infer the pressure and temperature history of upper-mantle rocks³⁵. The dependence is due to chemical reactions involving aluminous pyroxene and a buffer mineral containing aluminium, such as, for increasing pressure, plagioclase, spinel and garnet. This reaction leads, at the interface between pyroxene and the buffer mineral, to an aluminium distribution from which the pressure and temperature values can be tentatively inferred. The aluminium diffusion coefficient in clinopyroxene has been measured using the $^{27}Al(p, \gamma)^{28}Si$ resonant nuclear reaction³⁶, to help quantify the kinetics of the mineralogical reactions occurring in the Earth's mantle and to validate fully the use of geothermobarometers (see Fig. 6b).

The above two examples are the first applications to Earth sciences issues of the systematic determination of diffusion coefficients for a wide variety of slowly diffusing species and geological materials. These data can be used to identify the processes governing other fundamental geological phenomena, such as the kinetics of diffusion-controlled metamorphic reactions.

Conclusions and future prospects

Despite having been applied only recently to the Earth sciences, analytical techniques based on high-energy ion beams have already proved their utility in contributing to the understanding of problems relevant to crustal and mantle mass transfer.

These techniques are being developed continually, and several of their analytical features are expected to improve in the next few years. Many nuclear reactions involving elements of potential interest in the Earth sciences have not yet been fully studied and, even among those that are well characterized, not all have been applied. Indeed, an improved knowledge of nuclear reactions is attainable for many elements of Earth sciences interest, such as, for example, alkaline earths and transition elements. The other expected improvements of these analytical tools will come from technical progress, including the use of microbeams³⁷ and time-of-flight spectrometers³⁸. Microbeams, which allow the

probing of micrometre-sized areas, would permit the direct characterization of individual phases in heterogeneous geological samples, if sufficient caution were taken to minimize the thermal effects that can occur when focusing the beam on such a tiny spot. Unfortunately, the improvements in detector sensitivity that would avoid such a difficulty are not expected soon. The use of time-of-flight spectrometry is expected to enhance noticeably the mass resolution of RBS for medium and heavy elements. In addition, it should increase the resolution for light elements detected by ERDA, particularly for the analysis of lithium, beryllium, boron and carbon isotopes, which would then be more practicable.

The approach described here can be extended to a number of other Earth sciences problems in which gradients of chemical composition induced by interfacial or bulk phenomena have a key role. A tentative list would include first, metamorphism³⁹ during which mineralogical reactions occur at solid-liquid, and in some cases solid-solid, interfaces. Second, the cooling of hot magmas originating from the upper mantle, either in the Earth's crust (plutonism) or at its surface (volcanism) involves the stepwise crystallization of phases. This leads to mineral assemblages characteristic of the chemical composition of the molten parent liquid, temperature and pressure, and hence to elemental partitioning between these phases (see, for example, ref. 40). Again, solid-liquid interfaces have a dominant role during such a complex phenomenon. Third, issues similar to those described for water-rock interactions can be found in the field of the low-temperature transformation of sedimentary rocks, one example being the transformation of limestone (calcium carbonate) into dolomite (mixed calcium-magnesium carbonate) as a result of dissolution/precipitation processes involving a magnesium-rich solution⁴¹. The recent extension of this concept of metasomatism to the high-temperature transformation of mantle rocks involving changes in chemical composition, also raises issues of great interest⁴². A final potential application derives from the important isotope exchanges (mainly for hydrogen and oxygen) that occur during silicate dissolution. This process should be fully characterized and taken into account in any use of light-element isotope ratios as geothermometers⁴³. □

J.-C. Petit is at Centre d'Etudes Nucléaires de Fontenay aux Roses, CEN-FAR, BP 6, 92265 Fontenay aux Roses Cedex, France. J.-C. Dran is at Centre de Spectrométrie Nucléaires et de Spectrométrie de Masse, Bat. 104-108, BP 1, 91405 Orsay, France. G. Della Mea is at the Dipartimento di Ingegneria, Università di Trento, 38050 Mesiano and Istituto Interuniversitario di Fisica della Materia, Unità di Padova, 35131 Padova, Italy.

- Benjamin, T. M., Duffy, C. J. & Rogers, P. S. Z. *Nucl. Instrum. Meth. phys. Res.* **B30**, 454-458 (1988).
- Amsel, G. & Lanford, W. A. *Rev. Nucl. Particle Sci.* **34**, 435-460 (1984).
- Della Mea, G., Dran, J.-C., Petit, J.-C., Bezzon, G. & Rossi-Alvarez, C. *Nucl. Instrum. Meth. phys. Res.* **218**, 493-499 (1983).
- Houlier, B., Jaoul, O., Abel, F. & Liebermann, R. C. *Phys. Earth planet. Inter.* **50**, 240-250 (1988).
- Chu, W. K., Mayer, G. W. & Nicolet, M. A. *Backscattering Spectrometry* (Academic, New York, 1984).
- L'Ecuyer, J., Brassard, C., Cardinal, C. & Terreault, B. *Nucl. Instrum. Meth.* **149**, 271-277 (1978).
- Doyle, B. L. & Peercy, P. S. *Appl. Phys. Lett.* **34**, 811-813 (1979).
- Clark, G. J. et al. *Nucl. Instrum. Meth.* **149**, 9-18 (1978).
- Brown, L. A. *Rev. Earth planet. Sci.* **12**, 39-59 (1984).
- Laursen, T. & Lanford, W. A. *Nature* **276**, 153-156 (1978).
- Arnold, G. W. & Petit, J.-C. *Nucl. Instrum. Meth.* **209/210**, 1071-1077 (1983).
- Dran, J.-C., Langevin, Y. & Petit, J.-C. *Nucl. Instrum. Meth. phys. Res.* **B1**, 557-568 (1984).
- Schott, J. & Petit, J.-C. in *Aquatic Surface Chemistry: Chemical Processes at the Particle-Water Interface* (ed. Stumm, W. 293-315 (Wiley, New York, 1987).
- Magonthier, M.-C. et al. *Proc. 2nd Int. Conf. Natural Glasses* (ed. Konta, J.) 57-64 (Charles University, Praha, Czechoslovakia, 1987).
- Crovisier, J.-L., Honnorez, J. & Eberhart, J.-P. *Geochim. cosmochim. Acta* **51**, 2977-2990 (1987).
- Alt, J. C. & Honnorez, J. *Contr. Mineral. Petrol.* **87**, 149-169 (1984).
- Magonthier, M.-C., Petit, J.-C. & Dran, J.-C. *Earth planet. Sci. Lett.* (in the press).
- Zielinski, R. A. *J. geochem. Exploration* **18**, 285-306 (1983).
- Petrovic, R., Berner, R. A. & Goldhaber, M. B. *Geochim. cosmochim. Acta* **40**, 537-548 (1976).
- Holdren, G. R. Jr & Berner, R. A. *Geochim. cosmochim. Acta* **43**, 1161-1171 (1979).
- Schott, J. & Berner, R. A. *Geochim. cosmochim. Acta* **47**, 2233-2240 (1983).
- Petit, J.-C., Della Mea, G., Dran, J.-C., Schott, J. & Berner, R. A. *Nature* **325**, 705-707 (1987).
- Petit, J.-C., Dran, J.-C., Paccagnella, A. & Della Mea, G. *Earth planet. Sci. Lett.* **93**, 292-298 (1989).
- Casey, W. H., Westrich, H. R. & Arnold, G. W. *Geochim. cosmochim. Acta* **52**, 2795-2807 (1988).

- Pederson, L. R., Baer, D. R., McVay, G. L. & Engelhard, M. H. *J. Non-Cryst. Solids* **96**, 369-380 (1986).
- Westrich, H. R., Casey, W. H. & Arnold, G. W. *Geochim. cosmochim. Acta* **53**, 1681-1685 (1989).
- Petit, J.-C. et al. *Nucl. Instrum. Meth. phys. Res.* **B32**, 498-503 (1988).
- Petit, J.-C., Dran, J.-C., Della Mea, G. & Paccagnella, A. *Chem. Geol.* **78**, 219-227 (1989).
- Casey, W. H., Westrich, H. R., Arnold, G. W. & Banfield, J. F. *Geochim. cosmochim. Acta* **53**, 821-832 (1989).
- Dran, J.-C., Della Mea, G., Paccagnella, A., Petit, J.-C. & Menager, M.-T. *Radiochim. Acta* **44/45**, 299-304 (1988).
- Guegen, Y. & Nicolas, A. *Rev. Earth planet. Sci.* **8**, 119-144 (1980).
- Bohlen, S. R. & Lindsley, D. H. A. *Rev. Earth planet. Sci.* **15**, 397-420 (1987).
- Kohlstedt, D. L. & Hornack, P. *Am. geophys. Un. Geodyn. Ser.* **4**, 101-107 (1981).
- Gerard, O. & Jaoul, O. *J. geophys. Res.* **94**, 4119-4128 (1989).
- Sautter, V., Jaoul, O. & Abel, F. *Earth planet. Sci. Lett.* **89**, 109-114 (1988).
- Gasparik, T. & Lindsley, D. H. *Rev. Mineral.* **7**, 309-335 (1980).
- Grime, G. W. & Watts, F. (eds) *Proc. 1st Int. Conf. Nuclear Microprobe Technology and Applications*. *Nucl. Instrum. Meth. phys. Res.* **B30**, 227-502 (1988).
- Thomas, J.-P., Fallavier, M. & Ziani, A. *Nucl. Instr. Meth. phys. Res.* **B15**, 443-452 (1986).
- Rumble, D. III A. *Rev. Earth planet. Sci.* **10**, 221-233 (1982).
- Lemarchand, F., Villemont, B. & Calas, G. *Geochim. cosmochim. Acta* **51**, 1071-1081 (1987).
- Eugster, H. P. A. *Rev. Earth planet. Sci.* **8**, 35-63 (1980).
- Roden, M. F. & Murphy, V. R. A. *Rev. Earth planet. Sci.* **13**, 269-296 (1985).
- Bottinga, Y. & Javoy, M. *Earth planet. Sci. Lett.* **84**, 406-414 (1987).

ACKNOWLEDGEMENTS. We thank W. Casey, R. C. Ewing and C. W. White for their reviews, O. Jaoul, P. Mazzoldi, V. Sautter and B. Velde for discussions, and G. Baudin, H. Bernas and M. Jorda for their interest in and support of our work. We also thank C. Rossi-Alvarez and G. Bezzon for help during the implementation of these techniques at the INFN-Legnaro, G. Egeni, I. Motti, V. Rudelo for assistance in operating the 7-MV and 2-MV accelerators at Legnaro and R. Rampazzo for drawing the figures.

Evidence from gravity data for focused magmatic accretion along the Mid-Atlantic Ridge

J. Lin*, G. M. Purdy*, H. Schouten*, J.-C. Sempere† & C. Zervas†

* Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

† School of Oceanography, University of Washington, Seattle, Washington 98195, USA

Gravity data from the Mid-Atlantic Ridge between latitudes 27°50' N and 30°40' N show that the accretion of magma at the ridge is focused at discrete centres along the spreading axis. Large positive gravity anomalies equivalent to reductions of almost 50% in crustal thickness are observed over non-transform discontinuities bounding spreading segments.

MID-OCEAN ridges are the diverging spreading boundaries between lithospheric plates at which new oceanic lithosphere is created. Information on the density structure of the ridge axis provides critical constraints on models of magmatic accretion and mantle dynamic processes at diverging plate boundaries. This information is particularly valuable at slow-spreading ridges, such as the Mid-Atlantic Ridge (MAR), where dramatic changes in sea-floor magmatic and tectonic processes have been observed along the ridge axis.

From December 1988 to January 1989, we carried out an extensive SeaBeam bathymetry, gravity and magnetic survey of a 300-km-long section of the MAR, including the Atlantis transform, between latitudes 27°50' N and 30°40' N (Fig. 1). This experiment was the second part of a two-leg investigation of the volcanic and magmatic accretion processes along the MAR between the Kane (23°45' N) and Atlantis (30°05' N) transforms. The tectonic setting of the MAR in this area has been established from high-resolution bathymetry¹. This portion of the MAR is spreading at relatively slow rates of 1.2–1.4 cm yr⁻¹ (half rate)². Our survey covers six spreading segments which are offset by the 68-km-long Atlantis transform near latitude 30°05' N and by prominent non-transform discontinuities near latitudes 28°15', 28°42', 28°51' and 29°23' N (Fig. 2a). The morphology of the Atlantis transform is characterized by a narrow strike-slip zone within a deep transform valley that abuts against the ridge segments. In contrast, the offsetting ridge segments at the non-transform discontinuities are not connected by a narrow strike-slip fault zone but by oblique fault-bounded extensional basins. These non-transform discontinuities are associated with off-axis traces of fossil basins (Fig. 2a), indicating their persistence with time. Gravity values were recorded along ship tracks using a high-precision BGM3 gravimeter³ on board the research vessel *Robert D. Conrad*.

Results of this gravity analysis reveal that magmatic accretion on the MAR is highly focused at discrete centres along the ridge axis. Large gravity anomalies, equivalent to variations in crustal thickness of more than 3 km, are observed over spreading segments. Non-transform discontinuities, in addition to the Atlantis transform, are found to be associated consistently with discontinuities in sub-seafloor density structure. This provides clear evidence that minor non-transform discontinuities along the MAR rift valley do not just represent surficial, *en echelon* cracks

in the crust responding to external stresses, but are fundamental divisions of the magmatic, crustal-generation processes.

Anomalous features of gravity field

The free-air gravity anomaly is dominated by the attraction of sea-floor topography. Anomaly lows are associated with the greater depths of the rift valley and the 100°-striking Atlantis fracture-zone valley near latitude 30°05' N (Fig. 2b). The lowest anomalies (less than -90 mgal) appear over the nodal basins at the eastern and western ridge-transform intersections. Discontinuities in the gravity low along the north-south-trending MAR median valley are clearly visible at latitudes 28°15', 28°42', 28°51' and 29°23' N. All are associated with morphological discontinuities in the median valley. Analyses of magnetic data suggest that a 14-km westward jump of the spreading axis between the Atlantis transform and latitude 29°45' N occurred about 0.4 Myr ago⁴. The free-air gravity, however, reveals no such discontinuity at latitude 29°45' N. Dominant positive anomalies appear over topographic highs to the north and south of the Atlantis transform and over the flanks of median valleys. The largest positive anomalies (more than 90 mgal) appear over the highest mountains at the eastern and western 'inside corners', which are bordered by the MAR axis and the active transform.

We subtract from the free-air anomalies the predictable components of the gravity field to produce a map of complete mantle Bouguer anomaly which can be related directly to the sub-seafloor density structure. These components include the attraction of sea-floor topography (water-crust interface), which can be estimated accurately from the three-dimensional SeaBeam bathymetry, and the attraction of relief on the crust-mantle interface, assuming constant crustal thickness and density. Negligible sediment cover (<10 m) exists in the survey area, so its contribution to the gravity field can be ignored. These calculations were carried out using the method described by Kuo and Forsyth⁵.

The complete mantle Bouguer anomaly map thus reflects the gravity field arising from density anomalies in the crust or mantle. The most striking feature in this map is the pattern of circular gravity lows centred on all six spreading segments (Fig. 2c), which resemble the 'bull's eye'-shaped gravity lows identified⁵ in the southern MAR at latitude 32°30' S. The most prominent of such gravity lows is associated with the morphologically well defined spreading segment immediately south of the Atlantis transform (segment 2). The centre of this low (-30 mgal) is located near the mid-point of the spreading segment. The least pronounced gravity low is associated with a short segment at latitude 28°45' N (segment 4).

Individual profiles across the strike of the spreading segments clearly reveal a strong increase in mantle Bouguer anomaly away from the spreading axis (Fig. 3). The rate of increase is comparable to the expected effects of density increase in an ageing and cooling lithosphere. A three-dimensional lithospheric thermal model⁶ was used to calculate reference gravity fields (Fig. 3). This model assumes a mantle flow field that is induced solely

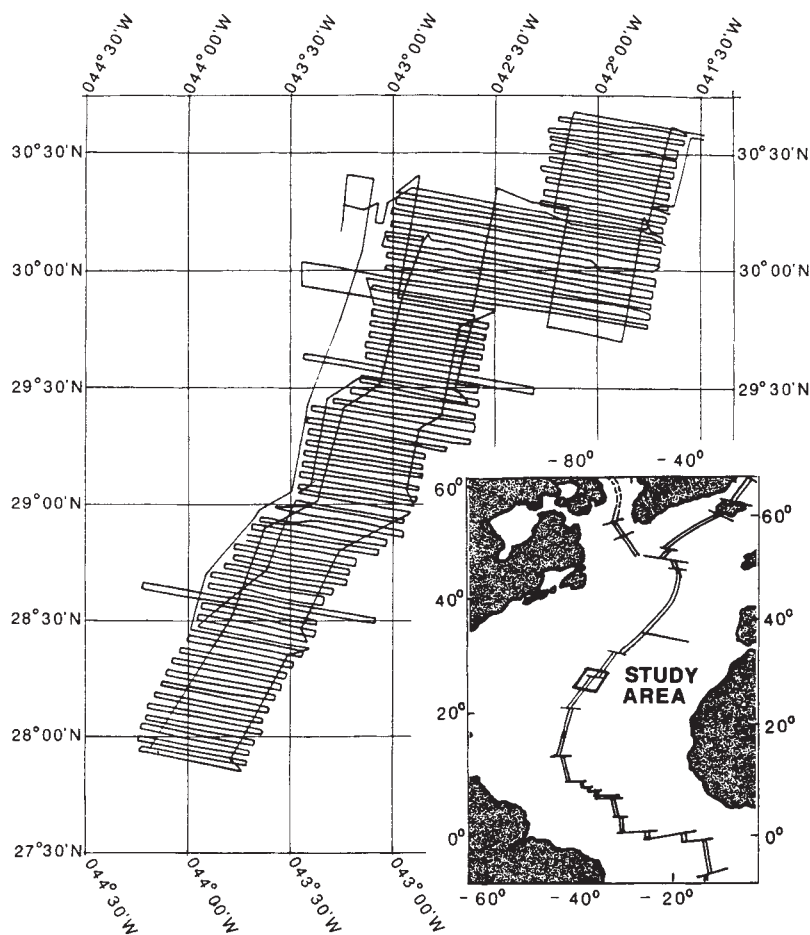


FIG. 1 Location map of survey area. Ship's track denoted by solid lines, along which SeaBeam bathymetry, gravity, and magnetic data were collected. The single and double lines on the index map (inset) represent major fracture zones and spreading centres, respectively.

by the divergence of two surface plates, separating at a constant half speed of 1.4 cm yr^{-1} and passing by each other at the Atlantis transform and the four discontinuities of small offset. A primary feature of the flow induced by plate separation is the relatively uniform mantle upwelling beneath spreading segments, except near large plate-boundary offsets^{5,6}. As a result, the calculated structure of the thermal boundary layer (lithosphere) is similar to that of a two-dimensional model except at the Atlantis transform.

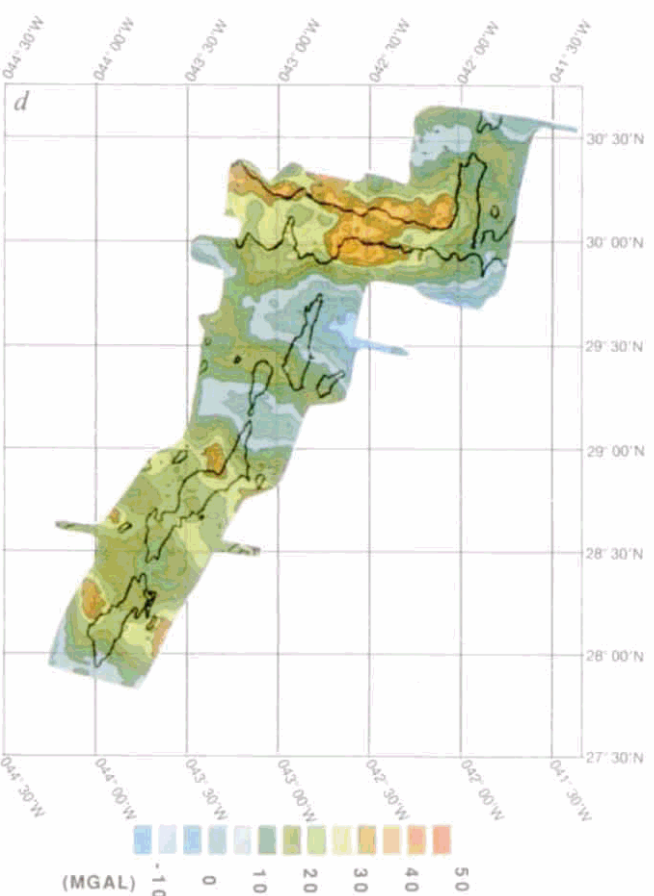
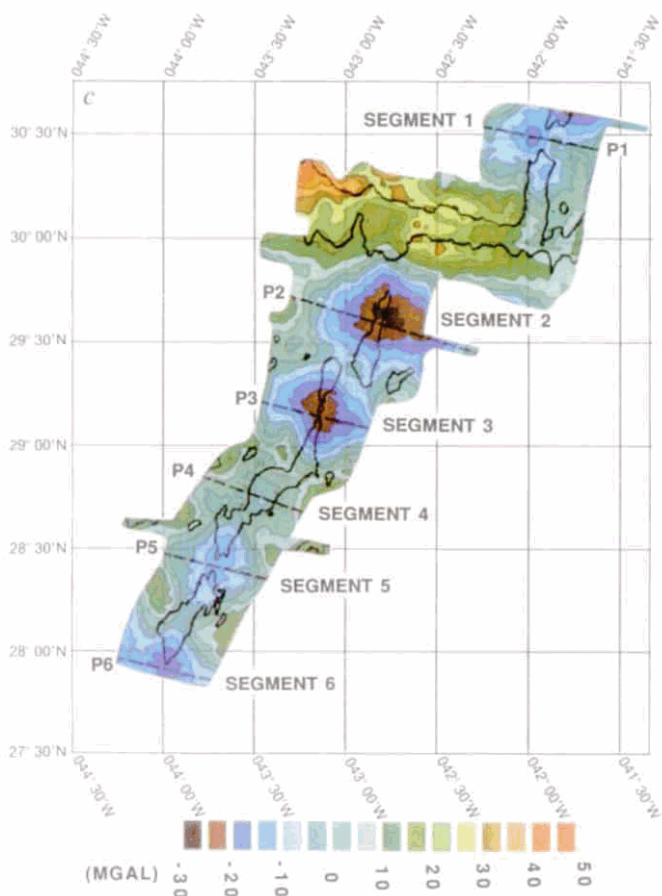
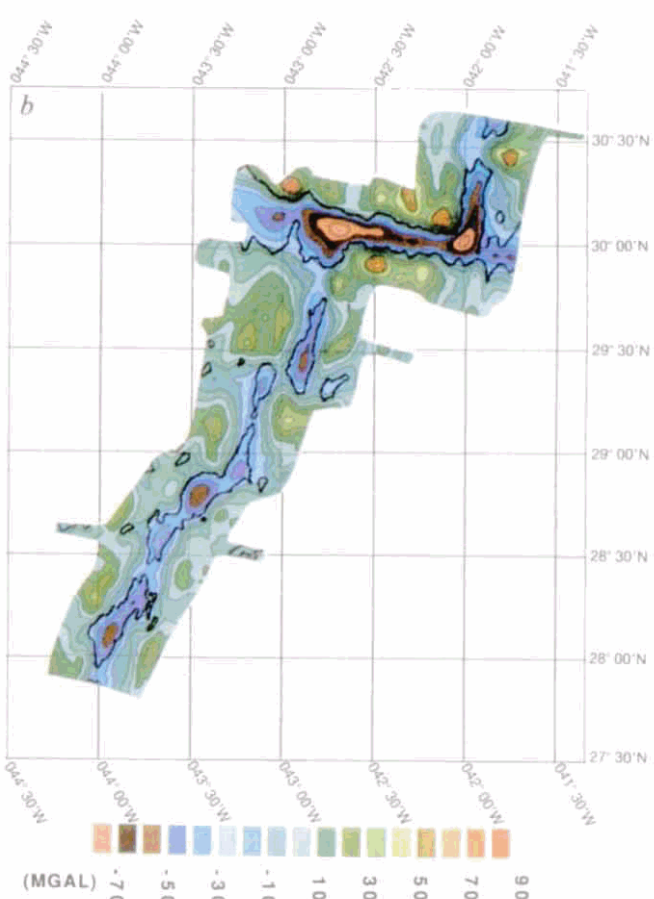
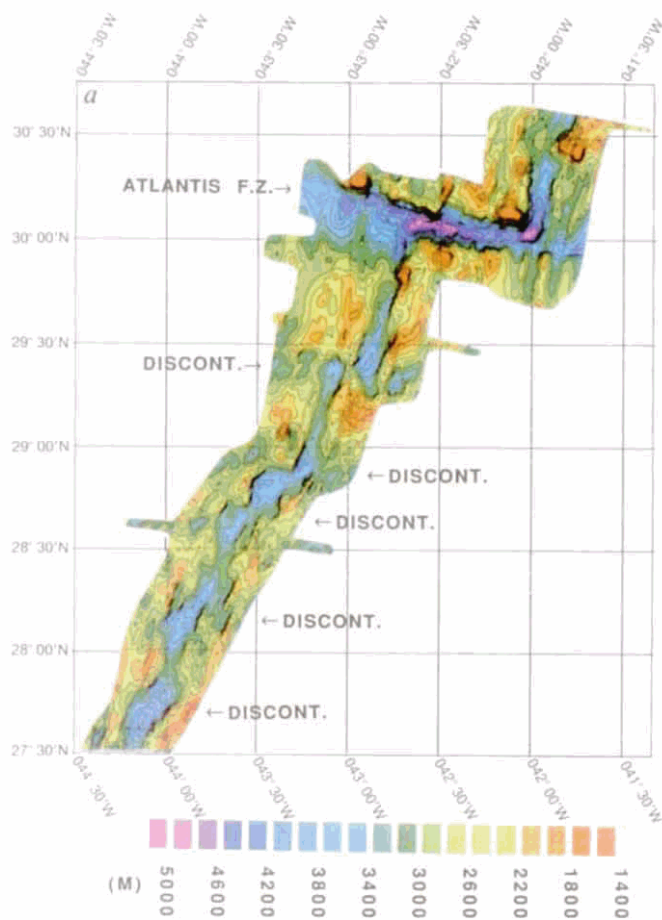
The most surprising results of these calculations are the dramatic changes in the observed mantle Bouguer anomaly along the ridge axis, which are not predicted by the thermal model of relatively uniform upwelling. The along-axis profile in Fig. 4a illustrates the striking difference between the observed and predicted gravity anomalies. The observed mantle Bouguer anomalies are consistently low at the mid-points of spreading segments and are lowest at segments 2 and 3; the intervening anomaly highs are always associated with the ends of segments. The anomaly is most positive at the Atlantis transform. In contrast, the thermal model of relatively uniform upwelling predicts significant increases in gravity only near the Atlantis transform at a rate half of the observed value. Near the discontinuities of small offset, the gravity variations predicted by the model of ref. 6 are insignificant. A map of residual anomaly is shown in Fig. 2d, and reflects the difference between the mantle Bouguer anomalies (Fig. 2c) and the gravity field based on the model of ref. 6.

The remarkable diversity in morphology and gravity anomalies observed along the ridge axis appears to be related to segment length. In our survey area between latitudes $27^{\circ}50' \text{ N}$ and $30^{\circ}40' \text{ N}$, segment 2 is longest (75 km) and is associated with the lowest 'bull's eye'-shaped gravity anomaly (Fig. 2c). This is in sharp contrast to the weak gravity low associated with

segment 4, which is only 18 km long. Lower values of mantle Bouguer anomalies are observed more frequently at longer spreading segments (Fig. 5a).

The relief of the axial depth in the median valley also shows a correlation with segment length. High-resolution SeaBeam

FIG. 2 a, SeaBeam bathymetry map contoured at 200-m intervals. The original SeaBeam bathymetric data have been interpolated onto a 1-km grid and machine-contoured. SeaBeam bathymetry data south of latitude $27^{\circ}50' \text{ N}$ were collected in a previous survey¹. b, Free-air gravity anomaly map contoured at 10-mgal intervals. 3,200-m bathymetric contours are shown to outline the position of the Atlantis fracture zone and rift valleys. Standard Eötvös corrections were made to the raw gravity data³. The root-mean-square misfit of the data at 270 survey line crossing points is 1.8 mgal, with a standard deviation of 2.9 mgal. The map was constructed by interpolating the data along ship tracks onto a grid spaced at 1 km and by filling the gaps with data extrapolated according to a minimum-curvature criterion. c, Mantle Bouguer anomaly map contoured at 5-mgal intervals. Zero level is arbitrary. Mantle Bouguer anomalies were generated by subtracting from the free-air anomaly the attraction of sea-floor topography and the attraction of relief on the crust-mantle interface. The two-dimensional fast Fourier transform (FFT) approach of Parker²⁶ was used for calculating gravity fields due to density interfaces. The nominal crust was assumed to be 6 km thick. Densities of 1.03, 2.7, and $3.3 \times 10^6 \text{ g m}^{-3}$ were assumed for sea water, crust and mantle respectively. Maps of density interfaces were interpolated and extrapolated into a rectangular array of 1-km spacing grids and mirrored on edges of the map to reduce edge effects to less than 2 mgal within 3 km. The locations of representative profiles shown in Fig. 3 are marked by dashed lines. d, Residual anomaly map contoured at 5-mgal intervals. Zero level is arbitrary. Notice that high anomalies exist near the Atlantis fracture zone and non-transform discontinuities at latitudes $28^{\circ}15'$, $28^{\circ}42'$, $28^{\circ}51'$ and $29^{\circ}23' \text{ N}$. This residual anomaly map is calculated by subtracting from the observed mantle Bouguer anomaly the gravity due to lithospheric cooling, based on the model of ref. 6.



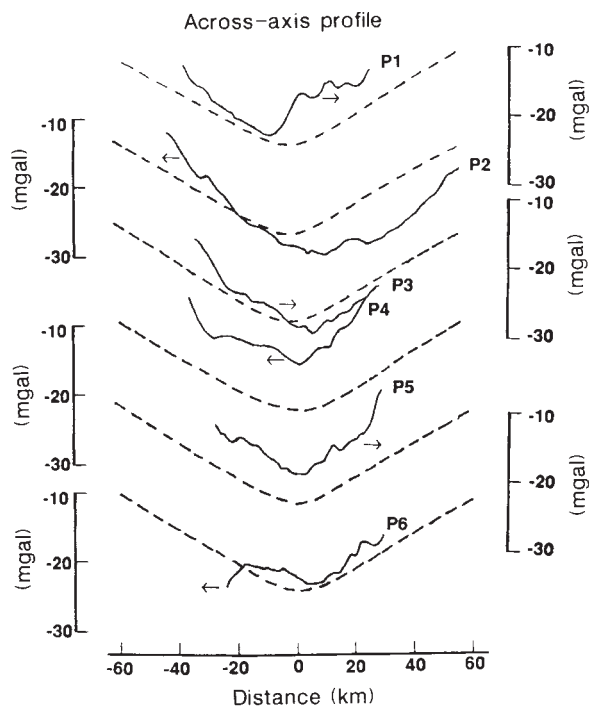


FIG. 3 A comparison between the observed anomalies (solid lines) and the predicted gravity resulting from lithospheric cooling for model of ref. 6 (dashed lines) at representative profiles across six spreading segments. Profile locations are shown in Fig. 2c. This three-dimensional thermal model assumes temperatures of 0 °C at the sea floor and 1,350 °C at a depth of 100 km. Temperatures were converted to density changes by multiplying by a thermal expansion coefficient of $3.4 \times 10^{-5} \text{ °C}^{-1}$, a value close to that estimated from sea-floor subsidence with age^{5,27}. We then calculate the gravity signals at sea level that result from these density changes.

bathymetric mapping of the MAR between the Kane transform (23°45' N) and the Atlantis transform (30°05' N) reveals that there are no other transforms in this 800-km portion of the spreading centre; this plate boundary is composed of 15 segments of varying length separated only by non-transform discontinuities¹. A compilation of data points for these 15 spreading segments clearly reveals that longer segments have greater topographic relief (Fig. 5b). This correlation has also been found

for spreading segments at other portions of the MAR and at other ridges⁷.

Models of sub-seafloor density structure

We postulate that the collective effects of significant along-axis changes in the crustal thickness and the density of shallow mantle are the primary sources of the strong mantle Bouguer anomalies along the MAR axis. We examine two limiting models in which the sole source of the residual gravity anomalies is assumed to be either changes in crustal thickness or variations in mantle density. Although the gravity data alone are insufficient to distinguish the direct contribution from each of these sources, the analysis of the data does yield valuable constraints on acceptable values in each model. The density structure constrained by the gravity data is consistent with a physical model in which both the melting of mantle rocks and the buoyancy-driven upwelling of mantle flows are highly focused beneath discrete centres along the MAR axis.

Source A: Along-axis variations in crustal thickness. The first model considers lateral changes in crustal thickness. To obtain an upper bound on the maximum possible changes in crustal thickness, we assumed that variations in crustal thickness were the sole source of the residual anomalies shown in Fig. 2d. The model crustal layer that results is consistently thicker beneath mid-portions of spreading segments and is thinner beneath ridge discontinuities (Fig. 4b). In segment 3, for example, the calculated crustal thickness ranges from 7.5 km at its centre to only 4 km at the southern end, nearly a 50% change in thickness. Because it has a greater length of thicker crust, the volume of crust generated over one million years at segment 3 would be eight times that at segment 4.

An important result from this crustal model is that significant crustal thinning occurs not only at the large offset Atlantis transform but also at the non-transform discontinuities of small offset. Magnetic data for the Atlantic basin show persistent disruptions in magnetic stripes at the off-axis traces of axial discontinuities^{8,9}. Seismic refraction studies at a few MAR discontinuities of small offset have shown that some are associated with crustal thinning^{10–14}. Our crustal model based on the gravity data provides independent evidence that non-transform discontinuities are fundamental boundaries of ridge magmatic segmentation.

Source B: Along-axis variations in mantle density. The second model considers lateral variations in the mantle density. We assume that each spreading segment is fed from below by a

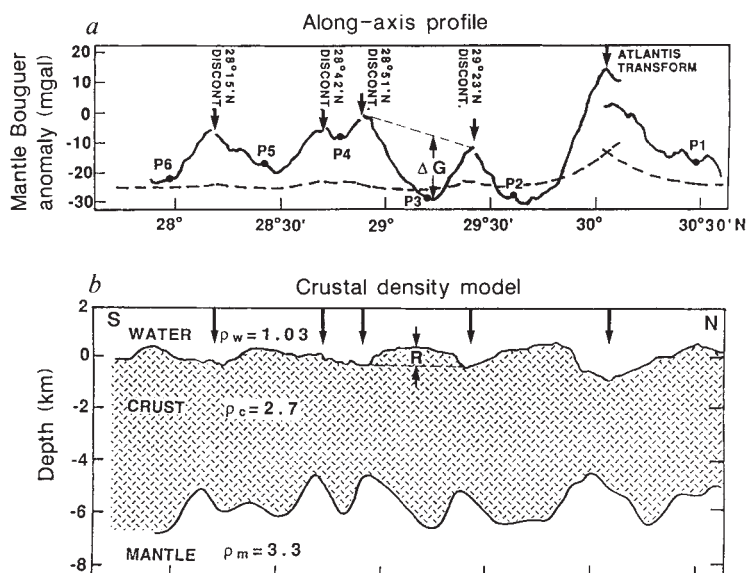
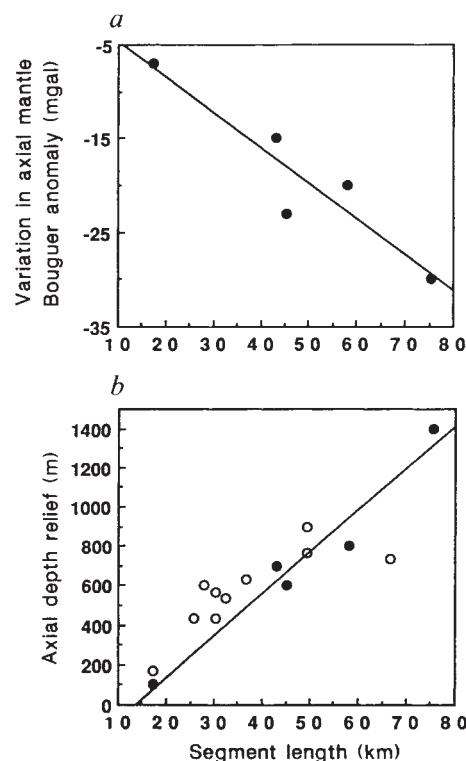


FIG. 4 a, Along-axis profile of mantle Bouguer anomaly (solid line) and predicted gravity of the model of ref. 6 (dashed line). Bold numbers denote crossing points with profiles of Fig. 3. ΔG is the change of mantle Bouguer gravity anomaly within a segment of length L . b, A cross-sectional view of the crustal structure along the MAR. Along-axis profile of sea-floor depth is shown by the upper boundary of the shaded region. R is axial topographic relief within a segment of length L . The theoretically calculated relief at the crust-mantle interface is shown by the lower boundary of the shaded region. The model of crustal density assumes that the residual gravity anomalies of Fig. 2d are due solely to lateral variations in crustal thickness. The residual gravity signal was continued downwards to the bottom of a 6-km crust and converted into crustal thickness assuming a crust-mantle density contrast of $0.6 \times 10^6 \text{ g m}^{-3}$. To prevent amplification of an unrealistic short-wavelength signal in the process of downward continuation, features with wavelength shorter than 25 km were filtered out.

FIG. 5 *a*, Plot of change in mantle Bouguer anomaly within a segment (ΔG) against segment length (L) for five segments in this survey area. Notice that longer segments are associated with lower gravity anomalies. *b*, Plot of axial topographic relief (R) against segment length L for 15 spreading segments between the Kane and Atlantis transforms. Filled circles correspond to the segments between latitudes 27°50' N and 30°45' N. Open circles correspond to segments between the Kane transform and latitude 27°50' N (ref. 1). Notice that longer segments have greater depth relief.



buoyancy-driven mantle plume which ascends from a zone of partial melt at depth (Fig. 6). This pattern of focused mantle upwelling has two direct effects on the mantle density structure. First, regions of ascending mantle flow are associated with negative density anomalies that result from anomalously high mantle temperatures as described by $\Delta\rho = -\rho_0\alpha\Delta T$, where ρ_0 is a reference density, α the coefficient of thermal expansion and ΔT the excess in mantle temperature. Second, additional negative density anomalies occur in the same regions because of the presence of residual mantle or small amounts of trapped basaltic melts (both are less dense than the unmelted parental mantle¹⁵). After 20% of melt extraction, for example, the density reduction in the residual mantle is equivalent to the thermal expansion that would be associated with a temperature increase of 500 °C.

If variations in mantle density were the sole source of the residual anomalies in Fig. 2*d*, we estimate that the along-axis mantle density changes would be as large as those associated with the lithospheric cooling across the ridge axis. In segment 3, for example, the required density anomalies are equivalent

to a 7-km thickening of the thermal boundary layer from the mid-point of the segment to its distal ends. We also notice that the residual anomalies for segments 2 and 3 are elongated in the direction of plate spreading, whereas that of segment 5 remains 'bull's eye'-shaped (Fig. 2*d*). If the residual anomalies are caused solely by thermally induced variations in mantle density, the mantle upwelling beneath segments 2 and 3 must take the form of two-dimensional convective rolls that strike in the direction of plate spreading. Alternatively, the mantle density anomalies at the ridge axis might be 'frozen' in the ageing lithosphere and be carried away from the ridge axis.

Implications for mechanisms of magmatic accretion

The density structure of the crust and that of the mantle are related by their common origin in mantle melting and upwelling. Beneath mid-portions of spreading segments, enhanced melting and the excess mantle temperature of the hypothesized ascending plume can cause negative density anomalies in the mantle. The same processes can produce excess amounts of melt which

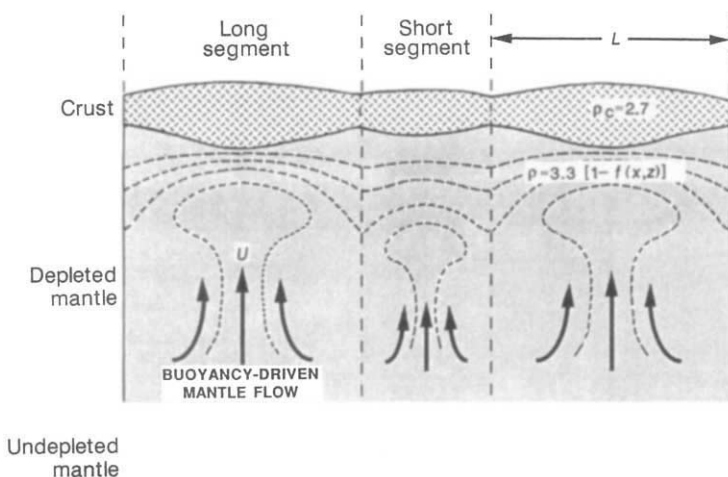


FIG. 6 The preferred model of MAR density structures that are consistent with the results of gravity analyses. We propose that the MAR spreading segments are fed from below by buoyancy-driven mantle flows. Thick solid lines with arrow heads represent mantle flows. Broken curves represent lines of equal density in the mantle. The average crustal density is $2.7 \times 10^6 \text{ g m}^{-3}$. The mantle density is $\rho = 3.3[1 - f(x, z)] \times 10^6 \text{ g m}^{-3}$, where $f(x, z)$ depends primarily on the mantle temperature, the amount of melt extraction and the amount of trapped basaltic melts. x and z are horizontal and vertical coordinates. Over regions of ascending mantle plumes, large temperature gradients and a high degree of decompression melting are expected. This could result in thicker crust and lower mantle densities beneath mid-portions of spreading segments. Both the changes in crustal thickness and variations in mantle density are likely to contribute significantly to the gravity field. A crustal model is shown in Fig. 4*b*, which assumes that the changes of crustal thickness are the sole source of the residual gravity anomalies.

migrate upwards through a porous mantle matrix, or through fractures and veins, to form thicker crust. It is therefore unlikely that significant density anomalies occur only in the crust or only in the mantle. Independent information on the crustal structure, obtainable from seismic studies for example, could be used to constrain the relative importance to the gravity field of each density structure.

It has been proposed that focused mantle upwelling is initiated by a Rayleigh–Taylor gravitational instability developed in the partial melt zone at depth which is overlain by heavier mantle material^{16–18}. We argue that, beyond the stage of initiation, it is the strong feedback between the melting and upwelling processes that maintains the stability of each mantle plume. Concentrated melting in regions of ascending mantle plumes can cause a reduction in the mantle viscosity^{16,19,20} as well as an increase in the mantle compositional buoyancy^{21–23}, and both changes favour further focusing of the mantle upwelling into regions of already existing melt. The mantle flow induced by the separation of rigid surface plates, on the other hand, may create horizontal shear stresses in the mantle in the direction of plate spreading. If these stresses are sufficiently large, the originally cylindrical plumes might be deformed into two-dimensional convective rolls oriented in the direction of plate spreading.

The thermal structure of a buoyancy-driven mantle plume depends in part on its geometric dimension: larger mantle plumes ascend faster. To illustrate this in a simple way, we assume that a mantle plume of diameter L is composed of a continuous series of buoyant spherical bodies (Stokes flow) with a density that is less than the surrounding mantle by $\Delta\rho$. The ascent velocity U of a buoyant spherical body is controlled by the balance between the upward buoyancy force, $\Delta\rho g[(4/3)\pi(L/2)^3]$, and the downward viscous drag exerted on the spherical surface, $6\pi\mu(L/2)U$, where g is the acceleration due to gravity and μ is the viscosity of the surrounding mantle. This gives $U = 2\Delta\rho g(L/2)^2/(9\mu)$, indicating that the ascent velocity is proportional to the square of the plume diameter.

For two-dimensional convective rolls, it can also be shown²⁴ that the ascent velocity of upwelling plumes is proportional to the square of the size of the rolls.

If longer spreading segments are associated with larger buoyancy-driven mantle plumes, we expect higher temperature gradients and a larger degree of decompression melting to occur beneath mid-points of longer spreading segments as the result of faster ascent velocities. This suggests that longer segments are associated with thicker crust and with more-negative mantle density anomalies and therefore more-negative gravity anomalies. This hypothesis is at least qualitatively consistent with the gravity data shown in Fig. 5a.

We suggest that both the transform and non-transform discontinuities along the MAR reflect fundamental boundaries between buoyancy-driven mantle plumes. In comparison with minor non-transform discontinuities, large-offset transforms are associated with more profound reduction in magmatic accretion because of the decreased mantle upwelling in the plate-separation-induced flow⁶. This explains the large positive mantle Bouguer anomalies observed over the Atlantis transform (Fig. 4) and the large depth relief of sea floor near large-offset transforms elsewhere at the MAR. Macdonald and others²⁵ have observed a similar relationship between the major transforms and minor discontinuities along the fast-spreading East Pacific Rise.

We conclude that magmatic accretion along the MAR is a fully three-dimensional process. This three-dimensional variability is characterized by focused magmatic accretion and mantle upwelling at discrete centres along the MAR axis. Non-transform discontinuities as well as transforms are the fundamental boundaries of ridge magmatic segmentation. We propose that the feedback between mantle melting and upwelling is an important mechanism in maintaining the stability of buoyancy-driven mantle plumes. The gravity data collected at the MAR provide critical constraints on models of MAR magmatic accretion and mantle dynamic processes. □

Received 4 December 1989; accepted 15 February 1990.

1. Sempere, J.-C., Purdy, G. M. & Schouten, H. *Nature* (in the press).
2. Klitgord, K. & Schouten, H. in *The Geology of North America* (eds Vogt, P. R. & Tucholke, B. E.) 351–378 (Geological Society of America, Boulder, Colorado, 1986).
3. Bell, R. E. & Watts, A. B. *Geophysics* **51**, 1480–1493 (1986).
4. Zervas, C., Sempere, J.-C., Lin, J. & Purdy, G. M. *Eos* **70**, 1035 (1989).
5. Kuo, B. Y. & Forsyth, D. W. *Mar. geophys. Res.* **10**, 205–232 (1988).
6. Phipps Morgan, J. & Forsyth, D. W. *J. geophys. Res.* **93**, 2955–2966 (1988).
7. Blackman, D. K. & Forsyth, D. W. *Earth planet. Sci. Lett.* **95**, 115–129 (1989).
8. Schouten, H. & White, R. S. *Geology* **8**, 175–179 (1980).
9. Schouten, H. & Klitgord, K. D. *Earth planet. Sci. Lett.* **59**, 255–266 (1982).
10. Mutter, J. C. & Detrick, R. S. *Geology* **12**, 534–537 (1984).
11. White, R. S., Detrick, R. S., Sinha, M. C. & Cormier, M. H. *Geophys. J.R. astr. Soc.* **79**, 779–798 (1984).
12. White, R. S. & Matthews, D. H. *Geophys. J.R. astr. Soc.* **61**, 401–435 (1980).
13. Sinha, M. C. & Loudon, K. E. *Geophys. J.R. astr. Soc.* **75**, 713–736 (1983).
14. White, R. S. *et al.* *Geology* (in the press).
15. Oxburgh, E. R. & Parmentier, E. M. *J. geol. Soc. Lond.* **133**, 343–354 (1977).
16. Whitehead, J. A., Dick, H. B. & Schouten, H. *Nature* **312**, 146–148 (1984).

17. Schouten, H., Klitgord, K. D. & Whitehead, J. A. *Nature* **317**, 225–229 (1985).
18. Crane, K. *Earth planet. Sci. Lett.* **72**, 405–414 (1985).
19. Auer, F., Beckmeier, H. & Oehlschlegel, G. *J. geophys. Res.* **49**, 89–92 (1981).
20. Buck, W. R. & Su, W. *Geophys. Res. Lett.* **16**, 641–644 (1989).
21. Rabinowicz, M., Ceuleneer, G. & Nicolas, A. *J. geophys. Res.* **92**, 3475–3486 (1987).
22. Scott, D. R. & Stevenson, D. J. *J. geophys. Res.* **94**, 2973–2988 (1989).
23. Sotin, C. & Parmentier, E. M. *Geophys. Res. Lett.* **8**, 835–838 (1989).
24. Turcotte, D. L. & Schubert, G. *Geodynamics* (Wiley, New York, 1983).
25. Macdonald, K. C. *et al.* *Nature* **335**, 217–225 (1988).
26. Parker, R. L. *Geophys. J.R. astr. Soc.* **31**, 447–455 (1972).
27. Parsons, B. & Sclater, J. G. *J. geophys. Res.* **82**, 803–827 (1977).

ACKNOWLEDGEMENTS. We are indebted to J. Phipps Morgan, B. Y. Kuo and D. Forsyth for allowing us to use their codes of gravity analysis. We thank the officers, crew and scientific personnel of the R/V *Conrad* (leg 2912) for their support at sea. W. Robinson reduced raw gravity data, B. Trams assisted with data collection at sea, and B. Y. Kuo assisted with data analysis. We benefited from discussions with E. M. Parmentier and J. Phipps Morgan and reviews by J. Fox, M. Kleinrock, D. Smith and M. Tivey. This work was funded by the NSF and a WHOI Culppeper Young Scientist Award (J.L.).

Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein

D. B. Carter^{*}, M. R. Deibel, Jr.[†], C. J. Dunn[‡], C-S. C. Tomich^{*}, A. L. Laborde[§], J. L. Slightom^{*}, A. E. Berger^{||}, M. J. Bienkowski^{||}, F. F. Sun[‡], R. N. McEwan^{*}, P. K. W. Harris^{*}, A. W. Yem[†], G. A. Waszak[†], J. G. Chosay[‡], L. C. Sieu^{*}, M. M. Hardee[‡], H. A. Zurcher-Neely[†], I. M. Reardon[†], R. L. Heinrichson[†], S. E. Truesdell[§], J. A. Shelly[§], T. E. Eessalu^{||}, B. M. Taylor[‡] & D. E. Tracey[‡]

Departments of ^{*}Molecular Biology Research, [†]Biopolymer Chemistry, [‡]Hypersensitivity Diseases Research, [§]Chemical and Biological Screening and ^{||}Cell Biology, The Upjohn Company, 301 Henrietta Street, Kalamazoo, Michigan 49007, USA

A human myelomonocytic cell line, U937, produced an interleukin-1 (IL-1) receptor antagonist protein (IRAP) which was purified and partially sequenced. A complementary DNA coding for IRAP was cloned and sequenced. The mature translation product of the cDNA has been expressed in *Escherichia coli* and was an active competitive inhibitor of the binding of IL-1 to the T-cell/fibroblast form of the IL-1 receptor. Recombinant IRAP specifically inhibited IL-1 bioactivity on T cells and endothelial cells *in vitro* and was a potent inhibitor of IL-1 induced corticosterone production *in vivo*.

INTERLEUKIN-1 (IL-1) has a key role in the body's response to infection, tissue injury, autoimmunity and inflammation^{1,2}. Two biochemically distinct forms of this cytokine, IL-1 α and IL-1 β , bind equally to IL-1 receptors to mediate these activities^{1,2}. The local and systemic activities of IL-1 are vital to a broad spectrum of immunological, endocrinological, haematological, neurological, and metabolic responses that occur in such pathological situations. In some cases, however, high levels of IL-1, often in concert with other cytokines such as tumour necrosis factor (TNF), lead to tissue destruction and chronic inflammation¹⁻³. Evidence exists for several natural feedback loops that may down-regulate IL-1 production or activity. One such loop involves the induction of adrenocorticotrophic and glucocorticoid hormones by way of IL-1 stimulation of the pituitary-adrenal axis⁴ and the subsequent inhibition by glucocorticoids of IL-1 production⁵ and activity, including interleukin-2 (IL-2) production by T cells⁶. Specific IL-1 functional inhibitors have also been described that may represent an alternative mechanism for the regulation of IL-1 activity. A specific inhibitor of IL-1-induced thymocyte proliferation and chondrocyte prostaglandin/collagenase release, which has a relative molecular mass (M_r) of ~22,000 (22K), has been found in culture supernatants of adherent human mononuclear cells stimulated with immobilized immune complexes⁷. Cloning and expression of a gene that apparently codes for this protein, termed IL-1ra, has been reported recently^{8,9}. A human urinary inhibitor of IL-1 activity with M_r 18-25K, which blocks IL-1 binding to its receptor on T-cells, has also been described¹⁰. More recently, an IL-1 receptor binding inhibitor of M_r ~23K (IL-1 INH) was shown to be released from cultured human monocytes after stimulation with granulocyte macrophage colony-stimulating factor (GM-CSF) (ref. 11).

We have purified an IL-1 receptor antagonist protein (IRAP) of apparent M_r ~25K from U937 human myelomonocytic cells treated with the differentiating agent phorbol myristate acetate (PMA) and stimulated with GM-CSF. The U937 cell line was

derived from a human histiocytic lymphoma and has phenotypic markers of a monoblast precursor of mononuclear cells¹². We obtained enough peptide sequence from the purified protein to clone the gene coding for the IRAP. Subsequent expression in *E. coli* of the cDNA coding for the mature translation product has provided large quantities of the recombinant IRAP for studies of IL-1 receptor binding activity and of *in vitro* and *in vivo* biological activities of the protein.

IRAP induction, purification and sequencing

Cell-free supernatants from cultured U937 cells were assayed for IL-1 antagonist activity using an IL-1 receptor binding assay (RBA) (ref. 13) with human YT-NCl cells and a sensitive bioassay, the 1A5 T-cell IL-1 antagonist assay⁶ (1A5 assay), which measures the reduction in IL-2 production by LBRM-33-1A5 murine thymoma cells stimulated with IL-1 (ref. 14). Unstimulated U937 cell supernatants did not contain measurable IL-1 inhibitory activity. Exposure of U937 cells to PMA for 48 h induced cell differentiation, as evidenced by increased adherence to culture vessel surfaces. These PMA-differentiated U937 cells produced low levels of IL-1 inhibitory activity which were increased at least 10-fold after stimulation of the cells for an additional 48 h with GM-CSF.

A single activity eluting from a size exclusion column with M_r ~25K was detected in supernatants from stimulated U937

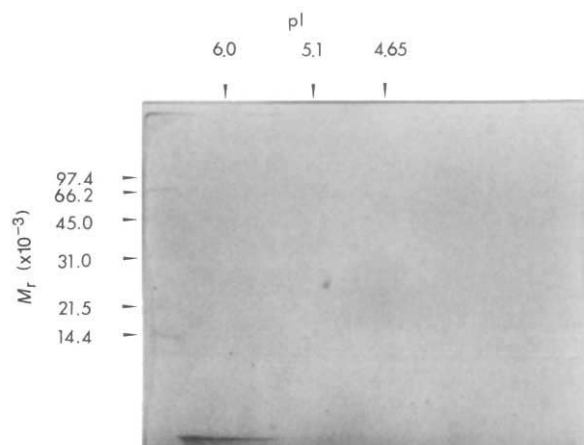


FIG. 1 Two-dimensional gel electrophoresis of purified U937 IRAP (fraction 6, Table 1). Two-dimensional electrophoresis was conducted using 1-mm diameter capillary-tube gels (7% polyacrylamide) in the first dimension (4-8 °C; 1,200 V, constant voltage) for 10-12 h. Ampholines having a pH range of 4-6 were used (Bio-Lytes, 2% w/v, Bio-Rad Laboratories). Focused sample gels were electrophoresed onto a second dimension of 18% polyacrylamide (9 cm $\times$ 7 cm) (0.1% SDS) according to the method of Laemmli³⁰. The gels were stained with Coomassie Brilliant Blue G-250.

FIG. 2 Nucleotide sequence of cDNA encoding U937 IRAP and the deduced amino acid sequence in the longest open reading frame. Nucleotide residues are numbered in the 5' to 3' direction. Deduced amino acids in the open reading frame are designated by their one-letter codes.

cells. In contrast, by isoelectric focusing in polyacrylamide gels, four peaks of inhibitory activity were observed between pI 4.9 and 5.5. Of these four forms, a species of pI 5.1 had the highest activity in both assays. Using these preliminary characteristics of the IRAP protein, a purification scheme was designed and implemented as shown in Table 1. The combination of size exclusion FPLC and HPLC with C4 reverse phase HPLC was sufficient to provide homogeneous native IRAP protein. The final preparation of IRAP was analysed by two-dimensional polyacrylamide gel electrophoresis (PAGE) to verify the purity of the IRAP protein; it gave a single Coomassie Brilliant Blue staining spot having the coordinates of pI 5.0 and M_r 25K (Fig. 1). After digestion of the purified IRAP with N-Glycanase/(Genzyme) under denaturing conditions, a protein band at M_r 22K was observed, indicating that the apoprotein possessed one or more N-glycosylation sites. The purified IRAP (Table 1, fraction 6) was S-pyridylethylated and digested with endoproteinase Lys C to generate peptides that were resolved by C18 reverse-phase HPLC. Several peptides were sequenced to provide the basis for the design of oligonucleotide probes with which to clone the IRAP gene.

Using degenerate oligonucleotides based on one of the IRAP peptides, KIDVVP, we plaque-purified several clones from a

NATURE · VOL 344 · 12 APRIL 1990

TABLE 1 Purification of U937 IRAP

No.	Fraction	Volume (ml)	Protein (mg)	Specific activity (inhibitory units mg^{-1})	Purification ($\times$)
1	Culture supernatant	7,400	1,590		
2	Ultrafiltration	34	877	0.323	1.0
3	Superose 12 FPLC-1	1.0	62.3	0.310	0.96
4	TSK Bio-Sil 125 HPLC-1	0.14	1.8	163	504
5	TSK Bio-Sil 125 HPLC-2	8.5	1.1	482	1,493
6	C4 RP HPLC	dry	0.07	3,222	9,975

U937 culture supernatants were produced, pooled and then subjected to five consecutive fractionation procedures. At each step, the volumes and protein contents were measured and the specific activities were measured in a 1A5 bioassay as previously described^{6,13}. Specific activities per mg protein were calculated such that one inhibitory unit (IU) gave 50% inhibition of one half-maximal thymocyte unit of rIL-1 β (ref. 29) in the 1A5 assay.

METHODS. U937 cell cultures were obtained from the American Type Culture Collection, Rockville, Md. Cells were cultured at 37 °C for 48 h at 5×10^5 cells ml^{-1} in RPMI 1640 medium (GIBCO), 7% fetal bovine serum (FBS, GIBCO), 2 mM L-glutamine, 100 U ml^{-1} penicillin, 100 $\mu\text{g ml}^{-1}$ streptomycin, 20 mM HEPES buffer, and 100 nM PMA (Sigma). After differentiation, the medium was removed and the cells, now adherent, were gently washed once with a small volume of Dulbecco's PBS. Cells were then cultured for an additional 48 h in RPMI 1640 containing 1% low-endotoxin FBS (HyClone), L-glutamine, penicillin, streptomycin, and rhGM-CSF (75 U ml^{-1} ; Amgen). Cell-free supernatants were collected, pooled and frozen at -20 °C. After thawing, phenylmethylsulphonyl fluoride was added to 0.2 mM final concentration. The solutions were concentrated by ultrafiltration with YM-5 and YM-10 filters (Amicon). Protein measurements were made with ISS Protein Gold (Enprotech) or the Bradford assay (Bio-Rad Kit) using BSA as the standard protein. Superose 12 FPLC-1: in 17 repetitive cycles, 2-ml aliquots of Fraction 2 concentrate were injected onto a Superose 12 prep grade FPLC column (1.6 $\times$ 50 cm) equilibrated with 0.1 M potassium phosphate, 1 M NaCl, pH 6.0. Fractions of 1 ml were collected at a flow rate of 1 ml min^{-1} . Appropriate fractions were collected and individually concentrated by Centricon-10 units (Amicon). After analysis by SDS-PAGE and bioassay, appropriate fraction concentrates were pooled and further concentrated to 1 ml by Speed Vac centrifugation (Savant). TSK Bio-Sil 125 HPLC-1: in 20 repetitive cycles, 50 μl of fraction 3 were injected onto a TSK-Bio-Sil-125 HPLC column (7.5 $\times$ 600 mm), using the same buffer system as described above. Fractions of 200 μl were collected. Appropriate fractions were pooled and concentrated by Centricon-10 filter units and finally by Speed Vac centrifugation as described previously. TSK Bio-Sil 125 HPLC-2: in seven repetitive cycles, 20 μl of fraction 4 were injected onto the same column as described above. Active fractions were pooled to give a final volume of 8.5 ml. C4 reverse phase HPLC: appropriate fractions from the TSK Bio-Sil 125 run (fraction 5) were injected directly onto a C4 reverse phase column (4.6 $\times$ 150 mm). Proteins which remained bound to the matrix following an extensive wash with 0.1% trifluoroacetic acid (TFA) were desorbed using a linear gradient from 28 to 32% acetonitrile in 0.1% TFA over a period of 44 min at a flow rate of 1 ml min^{-1} (room temperature). Representative aliquots of each fraction or pool were removed, taken to complete dryness by Speed Vac centrifugation, and then re-solubilized in tissue culture medium in preparation for assay.

and 69 conservative amino-acid substitutions for a total shared similarity of 72%. The motifs TTATTTAT or ATTTA, which may be associated with messenger RNA stability, found in the 3'-untranslated region of IL-1 α and IL-1 β and of other mRNAs specifying inflammatory mediators^{16,17} were not found in the nucleotide sequence of the IRAP cDNA.

Expression of the cDNA in *E. coli* was carried out by placing the cDNA coding for mature IRAP, beginning at position 84, behind the *trp* promoter in an *E. coli* expression vector¹⁸. We used synthetic oligonucleotides to supply IRAP sequence from positions 84-214 and to add an initiation codon to the cDNA. On induction by tryptophan starvation, a 22K protein (apparent M_r by SDS-PAGE) was synthesized that did not appear in extracts from *E. coli* cells carrying the expression vector without the IRAP cDNA. When the *E. coli* extracts were assayed in either the 1A5 assay or the IL-1 RBA, the extracts containing the 22K protein were active. The recombinant IRAP (rIRAP) protein was easily extracted in high yield from the *E. coli* pellets

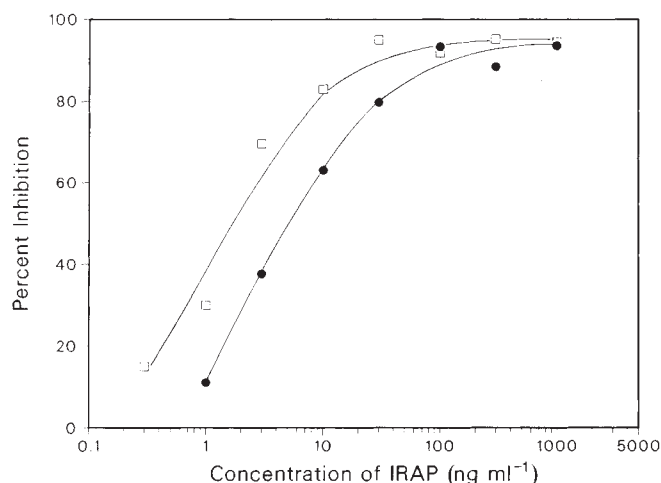
by mild hypotonic lysis to provide a protein at a purity greater than 80%. Homogeneous rIRAP was subsequently obtained by QAE Sepharose and TSK DEAE 5PW anion exchange chromatography steps, followed by size exclusion chromatography on a TSK Bio-Sil-125 HPLC column. A single spot having the coordinates of pI 6.0 and M_r 22K was observed (by two-dimensional PAGE). Edman degradation of the purified intact rIRAP gave the sequence NH₂-MRPSGRKSSKM, corresponding to that expected for IRAP preceded by a methionine.

rIRAP characterization

The bioactivity of rIRAP was measured in assays¹⁹ of IL-1-augmented human endothelial cell adhesiveness for human neutrophils and eosinophils. As shown in Fig. 3, rIRAP inhibited IL-1-induced adhesion for neutrophils and endothelial cells in a concentration-dependent fashion. With a specific activity of IL-1 β of 2×10^7 U mg^{-1} as a base (murine thymocyte assay half-maximal units), rIRAP had specific activities (one inhibi-

FIG. 3 Dose-response inhibition of IL-1-induced endothelial cell-leukocyte adhesion by rIRAP.

METHODS. Purified human blood neutrophils³⁶ (—□—), or eosinophils³⁷ (—●—) were labelled with ⁵¹Cr and suspended in Hanks balanced salt solution containing 1 mg ml^{-1} human serum albumin. Human umbilical vein endothelial cells (HUVEC) were cultured in Costar 48-well plates³⁸ and stimulated with rhIL-1 β (4 U ml^{-1}) for 4 h in the presence or absence of rIRAP. The HUVEC cultures were washed three times in culture medium (37 °C) to remove excess IL-1/rIRAP; leukocyte suspensions (neutrophil/eosinophil) were added to each well in a volume of 0.3 ml containing 4×10^5 cells and incubated for 30 min at 37 °C. Unattached leukocytes were gently washed off the plates using culture medium (37 °C); the remaining radiolabelled leukocytes adherent to the HUVECs were dissolved in SDS in 0.1 M NaOH and counted in a gamma counter to assess the degree of adhesion. The mean neutrophil adhesion in the absence of IL-1 was $8.12 \pm 0.84\%$ of total cells added and increased to $57.34 \pm 4.86\%$ after incubation of HUVEC with rhIL-1 β (4 U ml^{-1}); the mean eosinophil adhesion in the absence of IL-1 was $9.48 \pm 1.83\%$ of total cells added and increased to $48.24 \pm 2.45\%$ after incubation of HUVECs with rhIL-1 β . As shown in the graph, rIRAP caused a dose-related inhibition of neutrophil and eosinophil adhesion to HUVEC stimulated with IL-1.



tory unit (IU) gives 50% inhibition of one unit of IL-1) of 2.2×10^6 IU mg^{-1} and 7.5×10^5 IU mg^{-1} for inhibition of neutrophil and eosinophil adhesion, respectively. These differences may reflect different levels or types of IL-1-induced endothelial cell adhesion molecules responsible for binding to these two granulocyte populations.

The bioactivities of rIRAP in the 1A5 assay and murine thymocyte IL-1 co-mitogenesis assay²⁰ were also measured. In the 1A5 assay, rIRAP had a specific activity of $2.9 \pm 0.7 \times 10^4$ IU mg^{-1} compared with recombinant human IL-1 β (rhIL-1 β) and comparable activity against rhIL-1 α . In the thymocyte assay, however, rIRAP had a specific activity of $8.8 \pm 1.6 \times 10^5$ IU mg^{-1} . No inhibition by rIRAP of IL-2-induced thymocyte proliferation was seen. The specific activities of rIRAP were

comparable with those obtained with native IRAP against either rhIL-1 α or rhIL-1 β and complete inhibition of activity was seen at concentrations 10-fold above the respective IC_{50} values. The differences in rIRAP specific activities between the two assays may reflect differences in the numbers of occupied IL-1 receptors on 1A5 cells or thymocytes required for initiation of IL-2 production or IL-2 receptor expression.

Having established the activity of rIRAP in several *in vitro* assays, it was important to determine whether this protein inhibited IL-1-induced responses *in vivo*. To test this concept we assessed the effects of rIRAP on IL-1-induced corticosterone induction²¹ and neutrophilia²² in the mouse. The induction of raised plasma corticosterone levels and neutrophilia by intraperitoneal (i.p.) or intravenous administration of IL-1 in various species, including the mouse, has been well documented^{21,22}. We found that subcutaneous (s.c.) injection of rhIL-1 α in the mouse hind paw elicited dose-dependent increases in serum corticosterone levels, which peaked around 3 h and coincided with a profound blood neutrophilia; the suboptimal dose of 10^4 U rhIL-1 α /rhIL-1 β per mouse was selected to test the potential inhibitory activity of rIRAP on these two parameters 3 h after injection. Co-injection of rIRAP (300 μg per mouse) with rhIL-1 α s.c. in the hind paw completely abrogated the increase in serum corticosterone levels seen in mice treated with rhIL-1 α alone (Fig. 4a); maximum inhibition occurred between 30–300 μg rIRAP per mouse, whereas 50% suppression of corticosterone release (IC_{50}) was observed around 0.5 μg rIRAP per mouse (Fig. 4a). The approximate specific activity of rIRAP was 1.2×10^7 IU mg^{-1} in this assay. Similar effects were observed with rhIL-1 β , where doses of 0.3–300 μg rIRAP per mouse suppressed corticosterone production by 61–96%. Neutrophilia remained unaffected by rIRAP treatment (0.3–300 μg per mouse). Co-administration of rIRAP and rhIL-1 α at the same site was not essential for activity as a similar inhibitory effect was seen after i.p. injection of rIRAP in mice receiving s.c. rhIL-1 α (Fig. 4b).

The apparent selective suppression of corticosterone release induced by rhIL-1 α , compared with neutrophilia, by rIRAP treatment suggests a possible predilection of rIRAP for the IL-1 receptors in the pituitary and brain²³, as opposed to those required for IL-1-induced neutrophilia. Recent evidence for the existence of two distinct classes of IL-1 receptors supports this possibility. A high-affinity 87–100K IL-1 receptor has been identified on T lymphocytes and fibroblasts, in contrast to a 66–80K IL-1 receptor on murine bone marrow granulocytes, pre-B cells and macrophages, which has a slightly lower affinity^{24,25}. The K_D of murine pituitary and brain IL-1 receptors was found to be the same as for murine EL-4 6.1 thymoma cells²³, suggesting that the IL-1 receptors responsible for ACTH/corticosterone induction are of the T cell/fibroblast form. In order to investigate whether the differential *in vivo* activities of rIRAP could be related to selective inhibition of the T cell/fibroblast form of IL-1 receptor, we performed IL-1 receptor competition studies with rIRAP. The competition curves in Fig. 5 show that whereas rhIL-1 α and rhIL-1 β competed for the binding of radiolabelled rhIL-1 α to YT cells (bearing the T-cell/fibroblast form of IL-1 receptor) as well as to murine bone marrow cells, rIRAP only competed for IL-1 binding on YT cells. Thus, the lack of effect of rIRAP on IL-1 induced neutrophilia *in vivo* may reflect its inability to block the IL-1 receptors on bone marrow cells which include the precursor cells for peripheral blood neutrophils²⁶. Further support for the possibility that rIRAP may selectively bind to one class of IL-1 receptor was recently provided by the demonstration that IL-1ra blocked IL-1 binding to EL-4 thymoma cells, but not to 70Z/3 pre-B cells⁸. In addition, we found that rIRAP did not block IL-1 binding to 70Z/3 cells (Fig. 5 legend). Further studies are required to clarify the possible selectivity of IRAP IL-1 receptor interaction and its relationship to selective inhibition of IL-1 activities *in vivo*.

When the equilibrium binding constants for ^{125}I -rhIL-1 α or

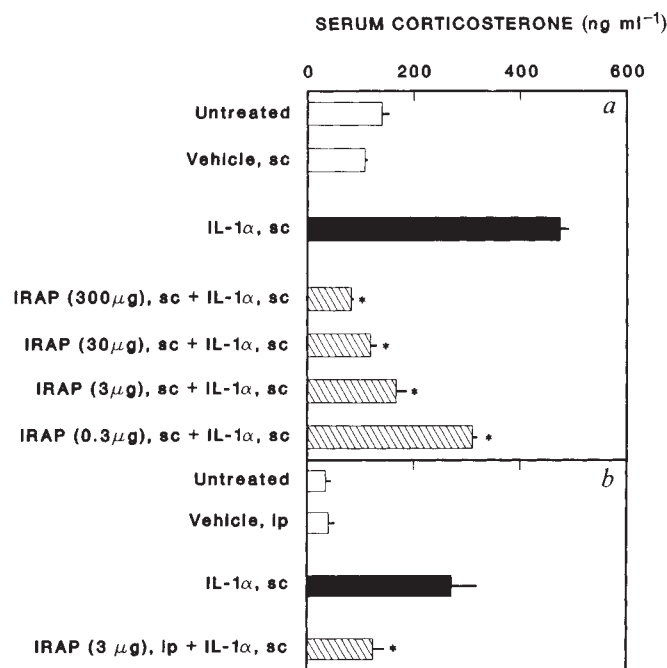


FIG. 4 a, Dose-related inhibition by rIRAP administered s.c. in the mouse hind paw of serum corticosterone levels induced by rhIL-1 α (10^4 U per mouse, s.c.). Dose-response studies indicated that 10^4 U rhIL-1 α (0.5 μg , 2×10^7 IU mg^{-1} specific activity determined in the murine thymocyte proliferation assay) induced suboptimal stimulation of corticosterone (63% of the level obtained with 10^5 U rhIL-1 α) and neutrophilia. Maximal corticosterone levels were attained between 2–3 h after s.c. injection of rhIL-1 α (10^4 U per mouse), with levels falling significantly by 4 h. Control, vehicle-injected mice showed no significant increase of corticosterone levels compared with non-injected normal mice at the three timepoints studied. The ratio of neutrophils to mononuclear leukocytes increased from a mean of 42:58 for control/vehicle-injected mice to 93:7 and 84:16 at 3 and 4 h respectively after rhIL-1 α injection. Neutrophil to mononuclear leukocyte ratios were unaffected by rIRAP treatment; rIRAP (0.3–300 μg per mouse) administered alone failed to alter normal ratios of corticosterone/neutrophil to mononuclear leukocytes or normal levels of corticosterone.

METHODS. All solutions were prepared in vehicle consisting of pyrogen-free sterile saline (0.9%) containing glycerol (10%) and injected s.c. in the plantar aspect of the mouse hind paw in a volume of 25 μl using a sterile 1 ml plastic syringe with a 27.5G stainless steel needle. The rhIL-1 α was obtained from Dainippon Pharmaceuticals Ltd. Endotoxin levels of rIRAP were determined to be less than 0.3 ng per mg of pure rIRAP. Male C3H/HeJ mice (20 gm body weight, Jackson Laboratories) were used throughout ($n=5$ mice per group); sera were assayed in duplicate for corticosterone using a radioimmunoassay kit (ICN Biomedicals). Blood smears were prepared from tail-vein puncture, fixed and stained using Diff-Quik (American Scientific); differential counts were performed recording the percentages of neutrophils and mononuclear leukocytes. b, Inhibition of rhIL-1 α -induced corticosterone production in the mouse by i.p. rIRAP. rIRAP (3 μg per mouse) was injected i.p. 30 min before rhIL-1 α (10^4 U per mouse, s.c.). * $P < 0.01$, treatment groups compared with groups treated with IL-1.

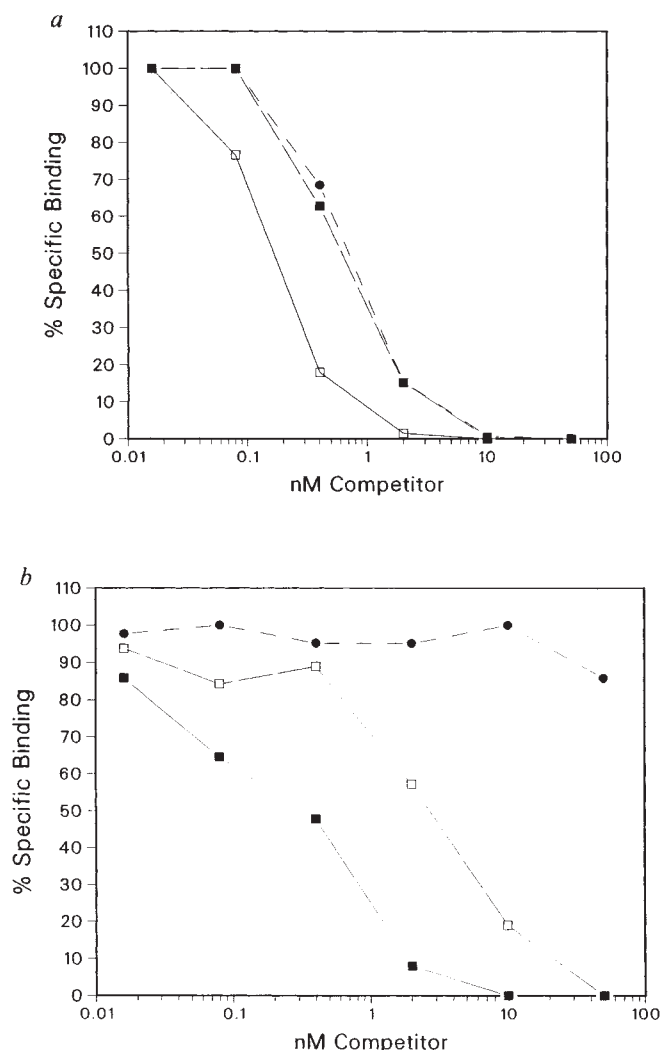


FIG. 5 Competition of IRAP for ^{125}I -IL-1 binding to IL-1 receptors. The per cent specific binding of ^{125}I -rhIL-1 α to a, YT-NCI cells and b, C3H/HeJ mouse bone marrow cells²⁶ in the presence of increasing concentrations of unlabelled competitors is shown. ■, Unlabelled rhIL-1 α ; □, unlabelled rhIL-1 β ; ●, unlabelled rIRAP. Similar results were seen using ^{125}I -rhIL-1 β , when the K_i values for rhIL-1 α , native IRAP and rIRAP binding to YT cells compared with ^{125}I -rhIL-1 β were 0.081, 0.29 and 0.23 nM, respectively. The specificity of IRAP competition was tested using a TNF-receptor positive human osteosarcoma cell line, G-292. Excess unlabelled rIRAP (20-fold molar excess) did not inhibit ^{125}I -rTNF from binding in a competition assay.

METHODS. Radiolabelling of rhIL-1 α and rhIL-1 β was performed as described previously^{39,40}. Receptor-binding assays were conducted in 96-well microtitre plates and contained 10^5 YT-NCI or 2.5×10^6 bone marrow cells, 250 pM ^{125}I -rhIL-1 α or 50 pM ^{125}I -rhIL-1 β and 0.016 nM to 50 nM unlabelled competitor proteins. Non-specifically bound counts were determined in the presence of 1,000-fold excess unlabelled ligand. The cells were incubated at 22 °C for 1 h and unbound radioactivity was removed by filtration. TNF-binding assays were done under conditions comparable to the IL-1 RBA. The IC_{50} of unlabelled competitor was calculated with a computer program using probit-log transformation to linearize competition curve data. K_i values were derived from IC_{50} values by the Cheng-Prusoff relationship⁴¹. To verify the applicability of this approach, Hill analysis of the data was performed by linear regression of the $\log [\% \text{ bound}/(100 - \% \text{ bound})]$ against $\log$ (inhibitor concentration) curve⁴¹. The slopes of the resulting lines were near 1, indicating that the conditions for valid application of the Cheng-Prusoff formula (single-site binding with no cooperativity) are met. In competition-binding experiments using the murine 70Z/3 pre-B cell line²⁵, unlabelled rhIL-1 α , rhIL-1 β and rIRAP affected ^{125}I -rhIL-1 α binding with results virtually identical to those shown with bone marrow cells.

^{125}I -rhIL-1 β were determined for cells expressing the T-cell form of the IL-1 receptor, such as YT cells, the K_D values were 0.074 nM and 0.050 nM respectively. Competition studies using radiolabelled IL-1 α and IL-1 β binding to YT cells were performed using unlabelled rIRAP or native IRAP at various concentrations. A concentration-dependent inhibition of specific radiolabelled IL-1 binding was seen, with K_i values for both IRAP proteins in the 0.2–0.3 nM range, nearly identical to each other and about 3–4-fold higher than for unlabelled IL-1 β (Fig. 5 legend). No inhibition by rIRAP of labelled TNF binding to its receptor was seen. Thus, rIRAP is a specific, high-affinity competitor of IL-1 binding to the T-cell/fibroblast form of IL-1 receptor and is indistinguishable from native IRAP in this regard. The concept of selective IL-1 receptor antagonism by IRAP raises several intriguing questions concerning IL-1 and IRAP binding to the two classes of IL-1 receptor. Distinct IL-1 receptor binding and triggering domains may exist on IL-1 α/β , but perhaps IRAP lacks the triggering domain. As IL-1 α/β and IRAP are structurally homologous and compete for one class of IL-1 receptor, they probably share a common domain responsible for receptor binding. The failure of IRAP to bind to IL-1 receptors on bone marrow and pre-B cells may indicate that distinct domains are responsible for binding of IL-1 α/β to the two classes of IL-1 receptors and that IRAP lacks one of these domains.

Evidence for the *in vivo* activity of recombinant IL-1ra (refs 8, 9), which seems to be identical to rIRAP, has been described in the rabbit²⁷; intravenous infusion of IL-1i (IL-1ra, 1–4 mg kg⁻¹) with rhIL-1 β (15 μg kg⁻¹) inhibited IL-1-induced hypotension and the induction of acute phase proteins. Monoclonal antibody specific for the murine T-cell/fibroblast IL-1 receptor suppressed responses elicited by IL-1 (acute inflammation; IL-6 production) in addition to lipopolysaccharide-induced acute inflammation and acute bacteraemia in which IL-1 seems to have an important role²⁸. These observations, together with the *in vitro* and *in vivo* data presented above, strongly suggest that antagonism of IL-1 binding to IL-1 receptors *in vivo* results in inhibition of a broad spectrum of pathophysiological responses associated with host defence and inflammation. Naturally induced IRAP may be a negative IL-1 feedback inhibitor whose role *in vivo* may be to regulate certain IL-1 functions. The exciting possibility that rIRAP may be a useful therapeutic agent to enhance the feedback mechanism for the treatment of inflammatory diseases remains to be tested. □

Received 15 January; accepted 13 March 1990.

- Dinarello, C. A. *FASEB J.* **2**, 108–115 (1988).
- Oppenheim, J. J., Kovacs, E. J., Matsushima, K. & Durum, S. K. *Immunol. Today* **7**, 45–56 (1986).
- Larick, J. W. & Kundel, S. L. *Pharm. Res.* **5**, 129–139 (1988).
- Besedovsky, H. del Rev. A., Sorokin, E. & Dinarello, C. A. *Science* **233**, 652–654 (1986).
- Snyder, D. S. & Unanue, E. R. *J. Immunol.* **129**, 1803–1805 (1982).
- Tracey, D. E., Hardee, M. M., Richard, K. A. & Paslay, J. W. *Immunopharmacology* **15**, 47–62 (1988).
- Arend, W. P., Joslin, F. G. & Massoni, R. J. *J. Immunol.* **134**, 3868–3875 (1985).
- Hannum, C. H. et al. *Nature* **343**, 336–340 (1990).
- Eisenberg, S. P. et al. *Nature* **343**, 341–346 (1990).
- Seckinger, P., Lowenthal, J. W., Williamson, K., Dayer, J. M. & MacDonald, H. R. *J. Immunol.* **139**, 1546–1549 (1987).
- Roux-Lombard, P., Modoux, C. & Dayer, J.-M. *Cytokine* **1**, 45–51 (1989).
- Harris, P. & Ralph, P. *J. Leukocyte Biol.* **37**, 407–413 (1985).
- Matsushima, K., Yodoi, J., Tagaya, Y. & Oppenheim, J. J. *J. Immunol.* **137**, 3183–3188 (1986).
- Gillis, S. & Mizel, S. B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1133–1137 (1981).
- Higgins, D. G. & Sharp, P. M. *Gene* **73**, 237–244 (1988).
- Caput, D. et al. *Proc. natn. Acad. Sci. U.S.A.* **83**, 1670–1674 (1986).
- Shaw, G. & Kamen, R. *Cell* **46**, 659–667 (1986).
- Olsen, M. K., Rodenbach, S. K., Curry, K. A. & Tomich, C.-S. C. *J. Biotech.* **9**, 179–190 (1989).
- Schleimer, R. P. & Rutledge, B. K. *J. Immunol.* **136**, 649–654 (1985).
- Gery, I., Gershon, R. K. & Waksman, B. H. *J. exp. Med.* **136**, 128–142 (1972).
- Morrissey, P. J., Charrier, K., Alpert, A. & Bressler, L. *J. Immunol.* **141**, 1456–1463 (1988).
- Ulich, T. R., del Castillo, J., Keys, M., Granger, G. A. & Ni, R.-X. *J. Immunol.* **139**, 3406–3415 (1987).
- Tracey, D. E. & De Souza, E. B. *Soc. Neurosci. Abst.* **14**, 1052 (1988).
- Bomsztyk, K. et al. *Proc. natn. Acad. Sci. U.S.A.* **86**, 8034–8038 (1989).
- Chizzonite, R. et al. *Proc. natn. Acad. Sci. U.S.A.* **86**, 8029–8033 (1989).
- Benjamin, W. R. et al. *J. Immunol.* **142**, 792–799 (1989).
- Ohlsson, K. et al. *Cytokine* **1**, 131 (1989).
- McIntyre, K. W. et al. *Cytokine* **1**, 150 (1989).
- Carter, D. B. et al. *Proteins: Structure, Function and Genetics* **3**, 121–129 (1988).
- Laemmli, U. K. *Nature* **227**, 680–685 (1970).

31. Sinha, N. D. *Nucleic Acids Res.* **12**, 4539–4557 (1984).
32. Lehrach, H., Diamond, D., Wozney, J. M. & Boedtker, H. *Biochemistry* **16**, 4743–4751 (1977).
33. Benton, W. D. & Davis, R. W. *Science* **196**, 180–182 (1977).
34. Leder, P., Tiemeier, D. & Enquist, L. *Science* **196**, 175–177 (1977).
35. Bosma, P. J., Van der Berg, E., Kooistra, T., Siemieniak, D. & Slightom, J. J. *biol. Chem.* **263**, 9129–9141 (1988).
36. Smith, R. J., Justen, J. M. & Sam, L. M. *Inflammation* **12**, 597–611 (1988).
37. Hensel, T. et al. *J. Immunol. Meth.* **122**, 97–104 (1989).
38. Jaffe, E. A., Nachman, R. L., Becker, C. G. & Minick, C. R. *J. clin. Invest.* **52**, 2745–2756 (1973).
39. Dower, S. K. et al. *J. exp. Med.* **162**, 501–515 (1985).
40. Dower, S. K. et al. *Nature* **324**, 266–268 (1986).
41. Titeler, M. *Receptor Pharmacology and Function* (eds Williams, M., Glennon, R. A. & Timmermans, P. B.) 17–46 (Dekker, New York, 1985).

ACKNOWLEDGEMENTS. We thank Dr F. Keszdy and D. Siemieniak for discussions on IRAP protein sequence, Dr N. T. Hatzenbuehler and M. Shea for DNA synthesis support, J. McGee for endotoxin measurements, J. Lynn for cell culture, R. F. Krzesicki for graphics support and K. J. Hiestand for preparation of the manuscript.

LETTERS TO NATURE

Blue stragglers at the centre of the post-core-collapse globular cluster NGC6397

M. Aurière*, S. Ortolani† & C. Lauzeral*

* Observatoire du Pic du Midi, F 65200 Bagnères de Bigorre, France

† Osservatorio Astronomico di Padova, Vicolo dell'Osservatorio 5, I 35122 Padova, Italy

THE nearby globular cluster NGC6397, 2.2 kpc from us, is an evolved cluster which has passed through core collapse^{1–3}; its core region is expanding after an earlier dynamical phase of contraction. Its proximity means that observations of it with CCD (charge-coupled device) detectors in arcsecond-seeing conditions simulate Hubble Space Telescope observations of more-distant objects, such as the archetypal post-core-collapse globular cluster M15, and are capable of testing the predictions of some recent models^{4,5} of post-collapse cluster dynamics. We have discovered in the central region of NGC6397 a population of 'blue stragglers'⁶, stars that are too hot for their luminosity in relation to subgiant stars. Five of these blue stragglers are within 0.07 pc of the centre of the cluster, and are brighter than the rest by one magnitude. We suggest that the blue stragglers are either coalesced stars resulting from stellar collisions expected to occur especially during core-collapse^{7,8}, or belong to a population of primordial close binaries that could provide the energy for the expansion of the core⁴.

Our observations were obtained at ESO (La Silla) on 19–20 March 1988 with a double-density RCA CCD at the 1.5-m Danish Telescope. The exposures were 60 s and 20 s and the FWHM of star profiles was 1.07'' and 0.95'' in the *B* and *V* passbands respectively. We used the same photometric package for stellar and surface photometry as in ref. 9, where details of the photometric technique can be found. Figure 1a shows an image of a 2' × 2' square field centred on NGC6397 in the *V* passband. Figure 1b shows contours of the 14'' × 14'' central region where several stars of the central clump¹ are well resolved with round images. Numbers correspond to blue straggler stars (BSs) in Table 1 and will be explained below. We used the equibarycentric method^{1,9} for resolved stars with *V* < 16 to determine the dynamical centre of NGC6397 and we found it less than 1'' away from star 1.

A power law can be fitted to the central part of the brightness profile of NGC6397, which is the signature of the post-core-collapse phase^{2,3}. CCD surface photometry of globular clusters is limited by sampling errors due to the statistical fluctuations of the bright end of the luminosity function. We used the star-stripping technique⁹ to reduce them when measuring the slope of the power law, subtracting numerically with Daophot bright stars which are resolved in the whole field studied. Fitting between 2.5'' and 1'' the *B* profile obtained after subtracting 29 red giants, we obtain a slope value of −0.60 with an error estimate of 0.1. This slope value is steeper than that obtained without star stripping, as there are no bright stars near the very centre of the cluster (the sky background was found to be <1 ADU per pixel on the *B* 1-min exposure and thus negligible). The

slope of the power law is similar to that found for M15 and NGC6624¹⁰, and is what is expected if there is a dynamically significant number of stars in the cluster with a mass 1.9 times larger than that of the luminous stars¹¹.

Figure 2a shows the colour-magnitude diagram (CMD) for 1,899 stars in the 150'' × 240'' field centred on NGC6397. A sequence of stars brighter and bluer than the turnoff is present at the location of the BSs. The BSs are on the extension of the main sequence, having the photometric properties of stars with a larger mass than that of the turnoff.

Thirty stars are found in the BSs region: *B* − *V* < 0.51 and 16.35 < *V* < 14.15. From examination of the CMD of ref. 12, we can estimate the number of field stars expected in our CMD. Normalizing to the field stars brighter than *V* = 16 and located to the red side of the giant branch (where they are more numerous), we find that we should not expect more than two

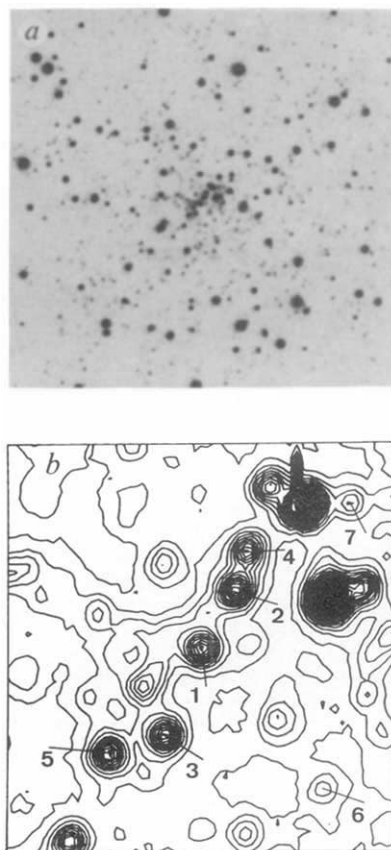


FIG. 1 a, The central region of NGC6397 in a 2' × 2' field in the *V* passband. FWHM of star profiles is 0.95''. North is up and east is to the left. There are no red giants at the centre and the central clump of stars is resolved into faint stars. b, Contours for the 14'' × 14'' central region in the *V* passband. One step is 60 ADU. Well resolved and measured BSs are numbered as in Table 1. The dynamical centre of the cluster is less than 1'' away from star 1.

TABLE 1 Blue stragglers in the central part of NGC6397

<i>N</i>	<i>R</i> (arcsec)	<i>V</i>	<i>B</i> - <i>V</i>
1	0.0	14.42	0.16
2	2.4	14.27	0.33
3	3.3	14.42	0.23
4	3.8	14.57	0.29
5	4.9	14.43	0.24
6	6.6	15.93	0.47
7	7.5	15.27	0.37
8	16.6	16.22	0.33
9	21.3	15.42	0.43
10	29.2	15.43	0.43
11	42.5	15.90	0.39
12	43.9	15.87	0.35
13	78.9	15.37	0.48
14	103.6	15.23	0.37

field stars in the BSs region of our CMD of Fig. 2*a*. In Table 1 we present the characteristics (distance to the centre in arcsec, magnitude and colour) of 14 well resolved BSs. The error on each measurement is dominated by that due to local crowding and we estimate that it is certainly less than 0.1 in both *V* and *B* - *V* for measurements presented in Table 1. One surprise from Table 1 is that several BSs are very near the centre of the cluster, and that the five BSs within the 6 inner arcsec (or 0.07 pc) are on average one magnitude brighter than the others ($\bar{V} = 14.42 \pm 0.10$ versus $\bar{V} = 15.62 \pm 0.36$). They are shown on Fig. 1*b*, and Fig. 2*b* presents the CMD for the inner 6" in radial distance.

There are three classical hypotheses on the nature of BSs⁶: that they represent single stars with extended main-sequence lifetime, coalesced stars, or mass transfer in binaries. However, in the case of globular clusters, it is generally concluded⁶ that BSs are more probably the result of physical coalescence in the case of stellar collisions^{7,8,13} (scenario 1) or of mass transfer in a primordial binary¹⁴ (scenario 2) rather than of unconventional processes in single stars. Both scenarios involve stars that will not grow into the red-giant phase after the turnoff stage but rather will become blue stars: they could explain the depletion of the red-giant branch and the central blueing already noticed in the central region of NGC6397¹ as well as in the other post-core-collapse clusters M15^{15,16} and M30^{17,18}.

We will now examine the two scenarios in more detail in order to understand more precisely the nature of the central

bright BSs in NGC6397 which have peculiar photometric properties and could play an important role in the dynamical life of the cluster. The five central BSs are on average about 2.2 magnitudes brighter than the turnoff ($V_{to} = 16.6 \pm 0.1$ from Fig. 1); using the classical mass-luminosity function for low-mass main-sequence stars (MSs)¹⁹ we find that they have the brightness of stars with about twice the mass at the turnoff. Scenarios 1 and 2 can lead to such chemically mixed, heavy stars^{13,14}.

Stellar collisions involving single or binary stars are expected to be numerous in the dense central regions of NGC6397, especially at the core-collapse phase, and thus scenario 1 is expected to occur^{7,8}. In this scenario, however, there is no obvious reason for obtaining only BSs with brightnesses of MSs with two turnoff-masses: one could expect to observe at the centre of NGC6397 brighter BSs (due to collisions of evolved stars with MSs⁷) or fainter ones (due to collisions of MSs with at least one fainter than the turnoff level; however, because of severe crowding, these fainter stars could be more difficult to detect at the centre than the brighter ones).

In scenario 2 we suggest that the BSs are primordial binaries that have not undergone physical coalescence of their two components and for which mass transfer has occurred. Although several uncertainties still remain with this scenario⁶ (including timescales, amount and rate of mass transfer) there are some supporting arguments. (1) Binaries composed of two MSs appear to exist in globular clusters in significant numbers²⁰ and a great number of galactic close binaries²¹ have a mass ratio near 1. (2) Because of mass segregation owing to two-body encounters, these stars concentrate in the central region²². During cluster evolution soft binaries are destroyed while hard ones shrink^{22,23}. The derived values for semimajor axis of the remaining binaries²³ are small enough to be compatible with mass exchange at the red-giant phase²⁴. Although the rate of shrinking slows rapidly as the semimajor axis decreases, some of the binaries could have coalesced²³. In the case of mass transfer (and of nearly equal-mass stars), however, the semimajor axis expands¹⁴, so that binaries with one component of the turnoff mass could have a better chance than others of escaping merging. (3) The five central BSs (one magnitude brighter than the others) should correspond to the maximum amount of mass transfer possible in a MS binary¹⁴ whereas the other BSs would correspond to smaller amounts. This difference in the amount of mass transfer could be due to shrinking of the orbit because of the increase of close encounters at the very centre of the cluster during core collapse: a smaller major axis will lead to a

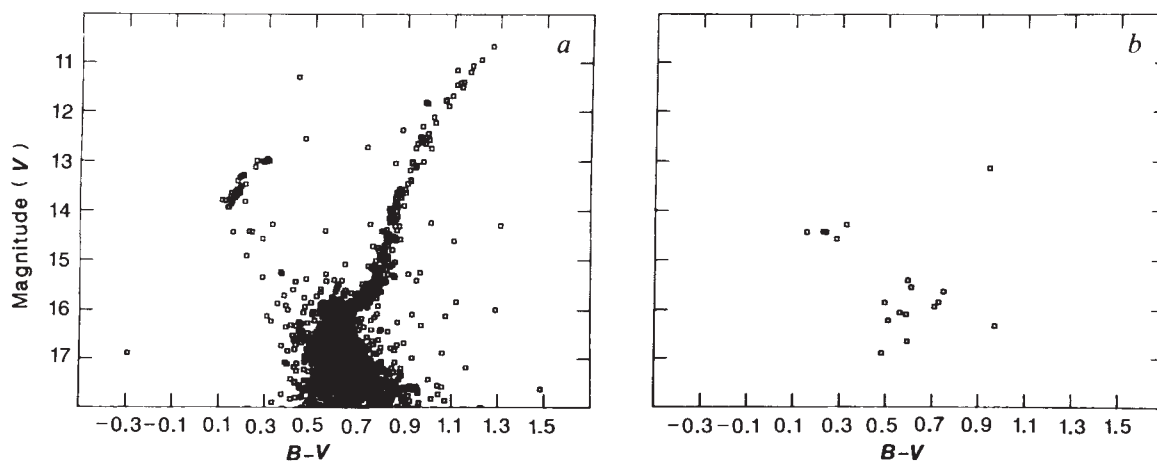


FIG. 2 Colour-magnitude diagram (CMD) for the stars in the central region of NGC6397. *a*, CMD for 1,899 stars in the complete $150'' \times 240''$ field. The characteristic giant and horizontal branches are well visible as well as the turnoff and the main sequence, which is widened because of crowding conditions. The scatter on the right of the giant branch is due to field stars.

On the blue side, as an extension of the main sequence, are the BSs. Only two field stars are expected in the BSs zone. *b*, CMD for the inner 6". There are no red giants but five bright BSs as well as some resolved main-sequence and subgiant stars.

greater mass exchange and a brighter BS²⁴.

The central group of five bright BSs makes zone 0–6" (zone 1) peculiar and deserves further comment. If the contribution of the five BSs is subtracted (the brightness of the MSs before collisions or mass transfer would be almost two magnitudes fainter) this zone would be significantly under-luminous with respect to the power law (which would give still a good fit in the region 6"–1'). We can thus associate zone 1 with what is expected for a resolved re-expanding core. The radius of zone 1, 0.07 pc, is of the order of magnitude predicted by models^{4,5} and almost equal to that found for NGC6752⁹, which strengthens our interpretation. In the two proposed scenarios, the observed BSs could be the luminous part of a population of heavy objects playing a dynamically significant role, as also suggested by the shallow slope of the power law of the brightness profile. In

scenario 2, our data would give observational support to the suggestion that primordial binaries could power the re-expansion of the core³.

We can now extrapolate our result to what could be observed in well studied but more-distant post-core-collapse globular clusters; 0.07 pc at a distance of 10 kpc, for example, corresponds to around 1", which is not easy but nevertheless possible to resolve from the ground. It is worth noting that for two post-core-collapse clusters, the closest 'stars' from the dynamical centre, namely AC214 in M15^{25,26} and CA195 in M30^{17,18} were bright blue objects and we suggest that they could be similar to zone 1 in NGC6397. Thus, projection of our result to more-distant post-core-collapse globular clusters appears promising and prompts us to observe them at sites where observations with good angular resolution can be obtained. □

Received 11 October 1989; accepted 23 February 1990.

1. Aurière, M. *Astr. Astrophys.* **109**, 301–304 (1982).
2. Djorgovski, S. & King, I. R. *Astrophys. J.* **305**, L61–L65 (1986).
3. Bailyn, C. D., Grindlay, J. E., Cohn, H. & Lugger, P. M. *Bull. astr. Soc. Am.* **20**, 966 (1988).
4. Goodman, J. & Hut, P. *Nature* **339**, 40–42 (1989).
5. Cohn, H., Hut, P. & Wise, M. *Astrophys. J.* **342**, 814–822 (1989).
6. Nemec, J. M. & Harris, H. C. *Astrophys. J.* **316**, 172–188 (1987).
7. Hills, J. G. & Day, C. A. *Astrophys. Lett.* **17**, 87–93 (1976).
8. Leonard, P. J. T. *Astr. J.* **98**, 217–226 (1989).
9. Aurière, M. & Ortolani, S. *Astr. Astrophys.* **221**, 20–26 (1989).
10. Lugger, P. M., Cohn, H., Grindlay, J. E., Bailyn, C. D. & Hertz, P. *Astrophys. J.* **320**, 482–492 (1987).
11. Lee, H. M. *Astrophys. J.* **319**, 772–800 (1987).
12. Alcaïno, G. et al. *Astr. J.* **94**, 917–947 (1987).
13. Benz, W. & Hills, J. G. *Astrophys. J.* **323**, 614–628 (1987).

14. McCrea, W. H. *Mon. Not. R. astr. Soc.* **128**, 147–155 (1964).
15. Aurière, M. & Cordoni, J. P. *Astr. Astrophys.* **100**, 307–310 (1981).
16. Bailyn, C. D., Grindlay, J. E., Cohn, H., Lugger, P. M., Stetson, P. B. & Hesser, J. E. *Astr. J.* **98**, 882–887 (1989).
17. Cordoni, J. P. & Aurière, M. *Astr. Astrophys. Suppl. Ser.* **58**, 559–564 (1984).
18. Piotto, G., King, I. R. & Djorgovski, S. *Astr. J.* **96**, 1918–1924 (1988).
19. Lang, K. R. in *Astrophysical Formulae* 545 (Springer, Berlin, 1980).
20. Pryor, C., McClure, R. D., Hesser, J. E. & Fletcher, J. M. in *Dynamics of Dense Stellar Systems* (ed. Merrit, D.) 175 (Cambridge University Press, 1989).
21. Halbwachs, J. L. *Astr. Astrophys.* **183**, 234–240 (1987).
22. Spitzer, L. Jr & Mathieu, R. D. *Astrophys. J.* **241**, 618–636 (1980).
23. Hills, J. G. *Astr. J.* **89**, 1811–1815 (1984).
24. Renzini, A., Mengel, J. G. & Sweigart, A. V. *Astr. Astrophys.* **56**, 369–376 (1977).
25. Aurière, M. & Cordoni, J. P. *Astr. Astrophys. Suppl. Ser.* **46**, 347–354 (1981).
26. Aurière, M., Le Fèvre, O. & Terzan, A. *Astr. Astrophys.* **138**, 415–420 (1984).

Frequency drift of 3-kHz interplanetary radio emissions: evidence of Fermi accelerated trapped radiation in a small heliosphere?

Andrzej Czechowski & Stanisław Grzedzielski

Space Research Centre, Polish Academy of Sciences, Orłowa 21, 01-237 Warsaw, Poland

NEITHER the termination shock wave formed where the solar wind ceases to be supersonic, nor the slightly more distant heliopause, where the wind runs into the interstellar medium, have been directly observed, but estimates based on observed cosmic-ray modulations and on pressure balance between the two media¹ suggest that they are 50–200 AU from the Sun. We argue here that the well-known interplanetary radio emission of 2–3 kHz in frequency^{2–4} is trapped in the electromagnetic cavity formed by the heliopause^{5,6}, and furthermore that the fluctuating solar wind will cause the frequency of this trapped radiation to increase at a rate dependent on the geometry of the cavity. Applying this interpretation to the previously unexplained frequency drift, amounting to ~ 1 kHz yr⁻¹, of the 3-kHz burst⁴, we estimate an average heliopause distance of 60–100 AU. This agrees with recent data from Pioneer 10 and Voyager 2, suggesting that the termination shock is located at a distance of ~ 50 AU, and implies that Voyager 1 may reach the shock in about 1993 and the heliopause as early as 1996.

Consider first the drift in frequency. We use the geometrical optics approximation which regards the signal as a classical particle with the hamiltonian $\omega = (c^2 k^2 + \omega_p^2)^{1/2}$ equal to the signal's frequency. The drift occurs because the local plasma density and hence plasma frequency $\omega_p(r, t)$ is time-dependent due to density fluctuations moving with the solar wind. Both the fluctuations with $\omega_p < \omega$ (through which the signal passes) and those with $\omega_p > \omega$ (from which it is reflected) contribute to

the drift. To find the net effect we performed computer simulations of this Fermi process assuming a spherically symmetric solar wind with the density ($\sim \omega_p^2$) profile $r^{-2}\alpha(r - Vt)$ where α consists of step functions (Fig. 1). The results confirm the idea that the contributions from $\omega_p < \omega$ events approximately compensate each other and that the net change in frequency comes from the reflection events. The latter are always 'head-on' collisions: that is why the frequency drift comes out as an effect of order V/c , not of order V^2/c^2 as in the case of radiation trapped in planetary magnetospheres⁷. The frequency gain per reflection is $\sim 2(V/c)\omega\eta$ where ω is the initial frequency and $\eta < 1$ a profile-dependent correction factor given by

$$\eta = P(1 - (\omega_-^2 + c^2 k_T^2)/\omega^2)^{1/2} \geq P(1 - (\omega_-^2 + c^2 k_T^2)/(\omega_+^2 + c^2 k_T^2))^{1/2} \quad (1)$$

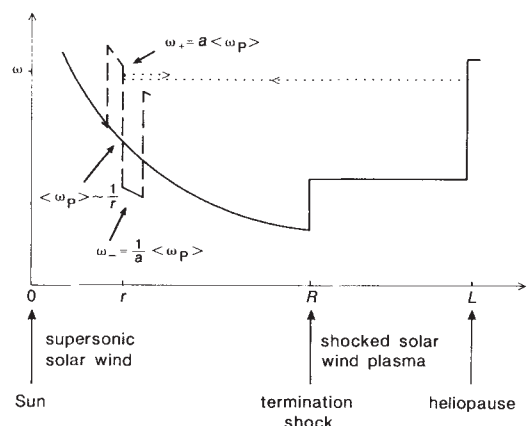


FIG. 1 Time-averaged plasma frequency ($\sim$ square root of the electron density) in the model heliosphere plotted against distance from the Sun. A fragment of the typical solar-wind profile assumed in computer simulations is shown by the dashed line, with the path of the signal of frequency ω reflecting from a moving step at r indicated by the dotted line. We assume that at the termination shock the average plasma frequency is doubled.

P describes the probability that the reflection occurs from a moving step in density and not from the flatter section in between; $\omega_+(\omega_-)$ are values of ω_p before (after) the step (we shall assume $\omega_+ = a\langle\omega_p\rangle$, $\omega_- = 1/a\langle\omega_p\rangle$ where $\langle\omega_p\rangle$ is the average local value) and k_T is the tangent part of the incident wave vector at the moment of reflection. For solar-wind density profiles observed by the Voyagers^{8,9}, $P \approx 1$ and $a \approx 2-4$ seem to be adequate.

Estimation of the actual drift rate is affected by two considerations. The cavity is not exactly spherical: at each reflection from the heliopause the angular momentum of the photon will change, leading to an even larger change in ck_T at subsequent reflection from the solar wind. Therefore, typical values of ck_T in (1) will be $\gg \omega_{+,-}$. The second point is that only the region inside the shock contributes significantly to the drift. We have calculated the drift in a simplified model, taking the cavity to be spherical (with the shock at R and the heliopause at L) but assuming that each reflection from the heliopause is a stochastic event with isotropic distribution of the final k ; we emphasize that a , R and L should be regarded as averages over all directions for a nonspherical cavity. The results for a 3-kHz signal do not deviate much from an approximate formula which holds if, close to the shock, the maximum ω_p of solar-wind fluctuations is $\ll \omega$:

$$\text{drift rate (kHz yr}^{-1}\text{)} = 10^6 (V/c) P (a^2 - 1/a^2)^{1/2} (n_e^E)^{1/2} \frac{\arcsin(R/L)}{(L/\text{AU})^2} \quad (2)$$

Here $n_e^E(\text{cm}^{-3})$ is the average electron density at 1 AU (we assume throughout that $n_e^E = 5 \text{ cm}^{-3}$).

The results (both numerical and equation (2)) show that there is an upper limit for the cavity size that can accommodate the observed drift of $\sim 1 \text{ kHz yr}^{-1}$. It is reached as $R \rightarrow L$ and equals $\sim 100 \text{ AU}$ (150 AU) for $a = 2(4)$ (numerical results). If R is not so close to L the cavity should be smaller: for example, $R = 50 \text{ AU}$ requires $L \approx 70(80) \text{ AU}$ for $a = 2(4)$. Although $a = 4$ can occur in corotating interaction regions, it is probably too high for a latitudinal average. Thus, the results for the drift rate of the 3-kHz signal favour the case of a small (60–100 AU) heliosphere. Recent Ly- α data from Pioneer 10 and Voyager 2^{10,11} show that at $\sim 50 \text{ AU}$ the hydrogen density starts to deviate sharply from the standard model values, a possible indication of proximity of shock. Voyager 1, with its present distance of 40 AU and a speed of $\sim 3.5 \text{ AU yr}^{-1}$, could thus reach the shock in about 1993 and the heliopause around 1996 (it moves in the upwind hemisphere, where the distance to the heliopause should be smaller).

A heliosphere this small requires high external pressure. In a proposed model¹² the excess pressure comes from the suprathermal particles accelerated at the shock and partially contained in a turbulent region bounding the (upwind) heliosphere. The turbulence provides also a way of compressing the interstellar plasma from the mean LISM density of $\approx 0.03 \text{ cm}^{-3}$ to densities $\geq 0.12 \text{ cm}^{-3}$ needed for confinement of the 3-kHz signal, although some of the photons would escape down low-density troughs expected in a turbulent region. The photon escape, either through the heliopause or down the tail of the heliosphere, may be the main cause of the observed fall-off of the 3-kHz signal, because according to our estimations¹³ the absorption by the medium is too weak to account for this, the reason being that in a non-spherical cavity the signal tends to keep away from high-density areas. Persistence of the observed drift for $\sim 6 \text{ months}^4$ (~ 300 reflection cycles) sets an upper limit for the escape rate.

Our model assumes that the photons constituting the 3-kHz signal come from an isolated emission burst. The data include also a weaker but perhaps more permanent⁴ ~ 2 -kHz component. If the heliosphere is small, the latter could be associated (in line with the earlier interpretations of the 3-kHz signal^{2,3,14}) with

the $2\omega_p$ emission from the termination shock placed at $\sim 42 \text{ AU}$ (upwind), which may be indicated by the Ly- α data quoted above. The lack of apparent drift could (W. S. Kurth, private communication) be caused by a sharp drop of instrumental sensitivity towards the nearby 2.4-kHz interference band. $\square$

Received 17 October 1989; accepted 14 February 1990.

1. Jokipii, J. R. in *Proc. XIXth ESLAB Symp. Les Diablerets* (ed. Marsden, R. G.) 375–387 (Reidel Dordrecht, 1986).
2. Kurth, W. S., Gurnett, D. A., Scarf, F. L. & Poynter, R. L. *Nature* **312**, 27–31 (1984).
3. Kurth, W. S., Gurnett, D. A. & Scarf, F. L. *Adv. Space Res.* **6**, 379–383 (1986).
4. Kurth, W. S., Gurnett, D. A., Scarf, F. L. & Poynter, R. L. *Geophys. Res. Lett.* **14**, 49–52 (1987).
5. Axford, W. I. *Solar Phys.* **100**, 575–586 (1985).
6. Fahr, H. J., Neutsch, W., Grzedzielski, S., Macek, W. & Ratkiewicz-Landowska, R. *Space Sci. Rev.* **43**, 329–381 (1986).
7. Barbosa, D. D. *Astrophys. J.* **243**, 1076–1087 (1981).
8. McNutt, R. L. *Geophys. Res. Lett.* **15**, 1307–1310 (1988).
9. Burlaga, L. F. *Space Sci. Rev.* **39**, 255–316 (1984).
10. Gangopadhyay, P., Ogawa, H. S. & Judge, D. *Astrophys. J.* **336**, 1012–1021 (1989).
11. Judge, D. L., Gangopadhyay, P. & Grzedzielski, S. in *Proc. 1st COSPAR Colloq. Warsaw 1989* (eds Page, D. E. & Grzedzielski, S.) (Pergamon, in the press).
12. Grzedzielski, S. & Ziemiakiewicz, J. *Adv. Space Res.* **9**, 247–250 (1989).
13. Czechowski, A. & Grzedzielski, S. in *Proc. 1st COSPAR Colloq. Warsaw 1989* (eds Page, D. E. & Grzedzielski, S.) (Pergamon, in the press).
14. Suess, S. T. & Dessler, A. J. *Nature* **317**, 702–703 (1985).

ACKNOWLEDGEMENTS. Helpful discussion with W. S. Kurth is gratefully acknowledged.

Structure, registry and imaging mechanism of alkylcyanobiphenyl molecules by tunnelling microscopy

D. P. E. Smith*, J. K. H. Hörber*, G. Binnig* & H. Neijoh†

* IBM Physics Group, Schellingstrasse 4, 8000 München 40, FRG

† IBM Yamato Laboratory, 1623-14 Shimotsuruma, Kanagawa 242, Japan

GRAPHITE is a popular choice as a substrate for the study of absorption of molecules on surfaces because of its relatively simple interaction with adsorbates and the ease of preparing a clean and homogeneous surface. Here we use scanning tunnelling microscopy (STM) to probe the graphite/liquid-crystal interface and we examine what we believe to be the first monolayer of the liquid with atomic resolution. We resolve the different functional groups of the molecules and determine their orientation and packing arrangement. By decreasing the tunnel-gap resistance we examine the underlying graphite substrate and deduce the registry of the adsorbed molecules with the graphite. We thus propose a microscopic picture of the adsorption and formation of the interfacial structure. We find that aromatically bonded carbon atoms are visible in the STM images whereas tetrahedrally- and triple-bonded carbon are invisible. Hydrogen and nitrogen atoms can also be seen, although with less apparent height than the aromatic carbon. Molecular orbital calculations predict electron densities in good agreement with the observed STM contrast.

The liquid crystal used in this study was 4'-n-alkyl-4-cyanobiphenyl (*mCB*, where $m = 8, 10, 12$ is the number of carbons in the alkyl group). These compounds exhibit a smectic liquid-crystal phase near room temperature, and previous STM studies have shown that the molecules form ordered layers on graphite^{1,2}. The thickness of the film is irrelevant for STM imaging because, as noted previously^{1,2}, the STM probe pierces through the bulk liquid and images only the liquid-solid interface. As *mCB* molecules are good insulators, we assume that in the STM images we observe only the first monolayer. For imaging, the samples must be maintained near their smectic-liquid to solid-crystalline transition temperature. If the samples are too warm, the films are completely liquid-like and no order is visible with the STM. On the other hand, if the films are cooled too far below their bulk solidification temperature they

no longer wet the graphite. There is a critical temperature range, approximately 10–20 °C below the bulk smectic transition temperature, where high-resolution STM imaging of well ordered molecular crystals is possible. As pointed out in the context of pure *n*-alkanes on graphite³, near the melting point the free energies of the liquid and solid are nearly equal and, in the vicinity of a surface, adsorption forces may stabilize a layer whose properties are roughly intermediate between the two.

The images shown here were taken with a tunnel bias of between 0.8 and 1.0 V (tip negative with respect to sample) and tunnel currents from 50 to 400 pA. Varying the tunnel bias between 0.6 and 1.2 V or changing the polarity did not alter the images significantly. Gap resistances below $\sim 5 \times 10^9 \Omega$ often produced distortions in the images which we interpret as mechanical perturbations of the weakly adsorbed molecules by the tip. When the gap resistance was reduced below $\sim 1 \times 10^7 \Omega$

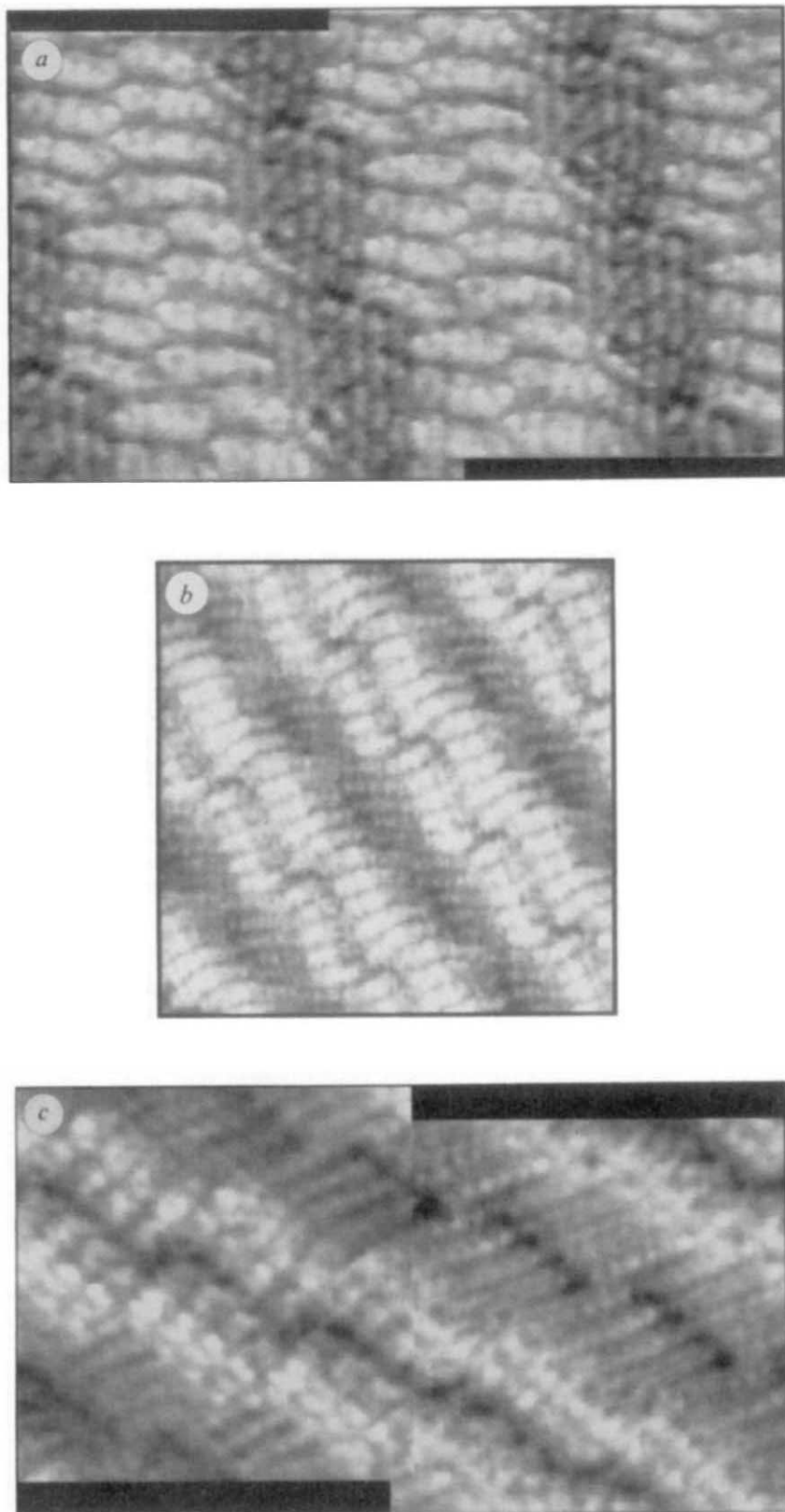


FIG. 1 *a*, Mosaic formed from two 56×56 -Å STM images of 8CB adsorbed on graphite. *b*, 112×112 -Å STM image of 10CB. *c*, Mosaic formed from two 56×56 -Å STM images of 12CB. The alkyl moiety of the molecules appears darker than the cyanobiphenyl moiety. The vertical scale from black to white corresponds to ~ 2 Å for all the images. The images were taken with a constant tunnel current of 100 pA and 0.8 V bias (tip negative). The samples were prepared by placing a few μg of *m*CB (BDH Ltd, Poole, England) on to freshly cleaved ZYA grade highly oriented pyrolytic graphite (Union Carbide Corp., Cleveland, Ohio) and heating above the liquid-crystalline to isotropic transition temperature. In the isotropic state *m*CB readily wets the graphite, forming a thin layer which quickly coats the surface. The samples were then allowed to cool. For 8CB (bulk transition temperature 21.5 °C) the samples were stored in a refrigerator for 24 h or more at 10 °C and then imaged at room temperature (22 °C). For 10CB (bulk transition 44 °C) the samples were imaged at 35 °C using a heated sample holder. 12CB (bulk transition 48 °C) was imaged at 40 °C. Tungsten wire, a.c.-etched in KOH, was used as the STM tip. Images were formed by keeping the tunnel current constant and monitoring the voltages on a piezoelectric tube scanner⁸.

the tip abruptly penetrated the molecular layer and the graphite substrate became visible. As we discuss below, determining the graphite orientation proved useful in deducing the registry of the molecules with the graphite.

Figure 1 shows STM images of 8, 10 and 12CB. As we have argued previously², the bright regions in Fig. 1 represent the locations of the cyanobiphenyl headgroups. The alkyl tails appear somewhat darker and contain structure which can be identified with individual methylene groups. The alkyl-cyanobiphenyls form a complex structure when they nucleate on graphite. The cyano groups point inwards towards each other and the alkyl tails radiate outwards from the headgroups in either a clockwise or an anticlockwise direction. Within a single crystalline domain the helicity of the tails is constant, although neighbouring domains may have different helicities. The cyano groups interdigitate slightly, thereby increasing the packing density. The molecules form unit cells composed of 8 molecules in the case of 8CB and 12CB and 10 molecules in the case of 10CB. Each unit cell is symmetric under rotation by 180°, thereby cancelling the electric dipole moments of the molecules within each unit cell.

The structure of 8CB presented in Fig. 1a is slightly different from that reported previously^{1,2}. Figure 1a shows a highly ordered structure that bears strong resemblance to the images of 10CB and 12CB (Fig. 1b, c). Earlier studies^{1,2} showed 8CB to have a more disordered and less tightly packed structure. We believe that this is because the present samples of 8CB were first cooled below their crystallization temperature. 8CB imaged at room temperature can show a variety of disordered phases⁴.

Figure 2a shows a particularly high-resolution STM image of 8CB in which bright pairs of rings are clearly observed in the locations expected for the biphenyl groups. Figure 3a is a 20×20-Å region from Fig. 2a, showing the packing of the cyanobiphenyl groups in a unit cell. A bright spot can be seen at the head of each biphenyl group which probably corresponds to the nitrogen atom. The large space between the phenyl group and the nitrogen atom suggests that the triple-bonded carbon atom in the cyano group offers low tunnel conductance. By comparing the STM images with molecular models, we conclude

that the bumps observable in the alkyl tails in Fig. 2a correspond to the locations of the hydrogen atoms rather than to the tetrahedrally bonded carbon atoms.

The two-dimensional lattice formed by the molecules is, in our opinion, primarily the result of registry of the alkyl tails with the graphite lattice. McGonigal *et al.*⁵ have similarly attributed the ordering of *n*-alkanes on graphite to registry with the substrate. As mentioned above, by piercing through the molecular film the directions of the graphite lattice vectors could be determined with respect to the alignment of the molecules. The results of these experiments showed that the alkyl chain of each molecule aligns along a graphite lattice vector and that the cyano groups of neighbouring molecules align along a different lattice vector (see Fig. 2b). Further evidence for registry occurs at grain boundaries, where the tails are often observed to rotate by 60°, matching the sixfold symmetry of graphite. For a variety of adsorbed atoms and molecules—such as He, Kr, N₂ and CH₄—the holes in the centres of the graphite rings are the energetically favourable sites⁶. To explain the high heat of adsorption of alkanes on graphite, Groszek⁷ has suggested that the hydrogen orbitals of *n*-alkanes fit into the holes in the (0001) plane of graphite.

To test these conclusions we have used CPK molecular models (Ealing Corp., South Natick, Massachusetts) on a lattice of holes with a spacing equivalent to $a_0 = 2.456$ Å to represent the graphite potential surface. The alkyl groups fit on to the lattice with very little strain, and when closely packed, the distance between chains, $\sqrt{3}a_0 = 4.25$ Å, agrees with the STM data to within 5%. The biphenyl groups show no particular registry. Tilting the carbon rings aids in the efficient packing of the molecules. We proposed previously² that the biphenyl groups stand completely on their edges. We now believe that at least for 8CB the biphenyls are tilted only slightly, in view of our ability to observe the phenyl groups as rings (Fig. 3b). When the cyano groups are interdigitated as far as possible without disrupting the overall packing of the headgroups, the dimensions of the molecular unit cell agree with our STM data reported previously². The models give the width of the unit cell to be 38.5, 44 and 47 Å for 8, 10 and 12CB, respectively; the STM

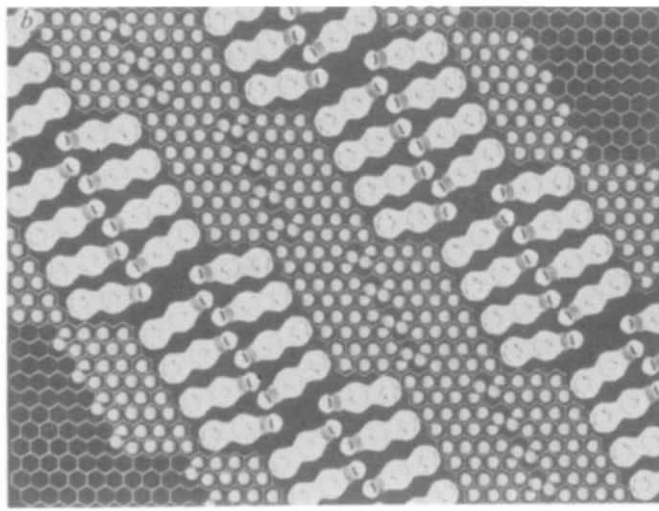
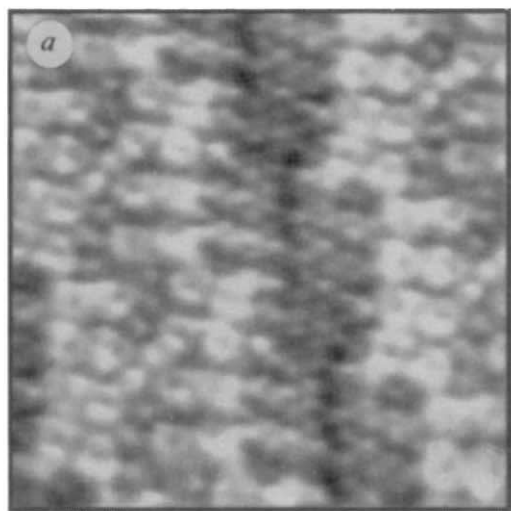


FIG. 2 a, 56×56-Å STM image of 8CB on graphite, showing pairs of rings suggesting the locations of the biphenyl groups. The tunnel current was 400 pA and the bias was 1.0 V (tip negative). b, Model of 8CB lattice showing the hydrogens of the alkyl tails registered with the centres of the hexagonal graphite lattice. The carbons in the alkyl groups and the hydrogens in the biphenyl groups are not shown. The headgroups are shown attached to the tails at angles of 12, 20, 28 and 35°. The overlap of neighbouring nitrogen atoms requires the cyano end of the headgroups to be spaced at least

5.5 Å apart. The registry of the alkyl tails with the graphite requires the alkyl end of the headgroups to be spaced only $2a_0 = 4.91$ Å apart. This difference introduces a deformation in the molecules which is visible as the fan-shaped structure of the headgroups. This explains why the molecular crystal is divided into unit cells and is not continuous. The molecules are forced to straighten out by the competing forces of alkyl registry and nitrogen repulsion. The accumulation of internal strain among the molecules means that it is energetically unfavourable to place too many molecules in a row.

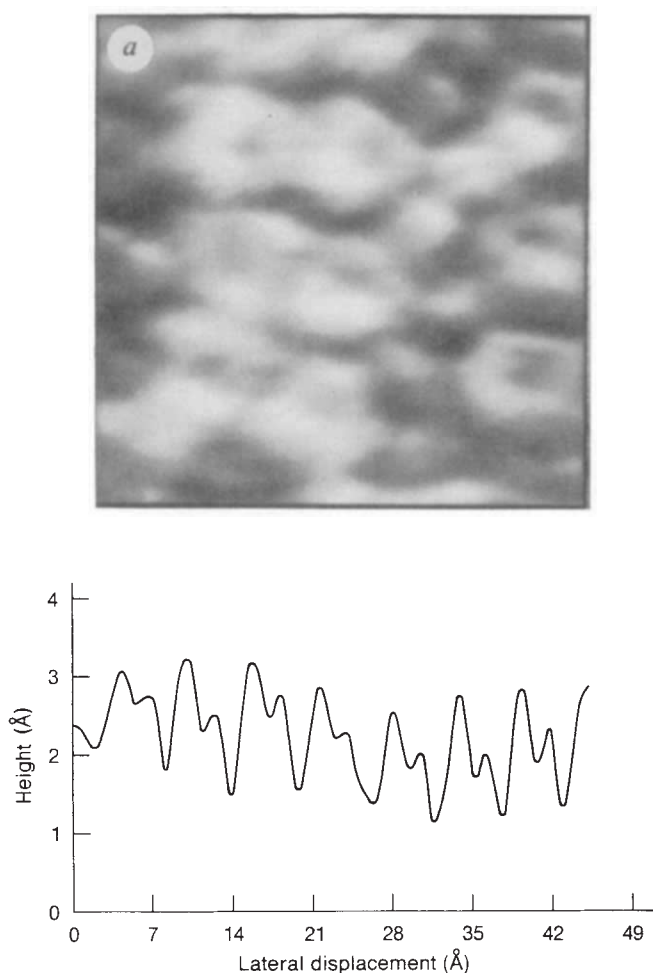


FIG. 3 *a*, 20×20 -Å area enlarged from the top right of Fig. 2*a*, showing in more detail the structure of the cyanobiphenyl headgroups. *b*, Contour taken through seven phenyl groups (from the right edge of Fig. 2*a*) showing the tilting of the carbon rings.

shows these widths to be 38, 43 and 47 Å. Our model for the 8CB lattice is presented in Fig. 2*b*.

The ring structures observed in Figs 2*a* and 3*a* suggest that aromatic carbon atoms play a large role in STM imaging, in contrast to triple-bonded and tetrahedrally bonded carbon. Aromatic groups always give the highest contrast in our STM images and their large apparent heights above the alkyl tails, sometimes as high as 3 Å, demonstrate that the contrast mechanism cannot be completely topographical in origin. The electronic nature of the STM contrast is confirmed by the good agreement of the STM experiments with the results of *ab initio* Hartree-Fock molecular orbital calculations (Fig. 4). STM is sensitive to electron density at the Fermi level rather than to total charge density⁸, so it is reasonable to concentrate on the highest occupied and the lowest unoccupied molecular orbitals (HOMO and LUMO). A similar approach has been used by Lippel *et al.*⁹ in the case of copper phthalocyanine adsorbed on copper. In our calculations the aromatic carbons and the nitrogen were found to have the highest LUMO and HOMO densities. The molecular orbital calculations suggest that the ease with which STM can differentiate the alkyl and cyanobiphenyl moieties is related directly to differences in their molecular orbital densities. This conclusion differs from that of Spong *et al.*¹⁰, who proposed that the STM contrast was due to changes in the substrate work function rather than to differences in the density of states. Our calculations also predict that the atomic force microscope¹¹,

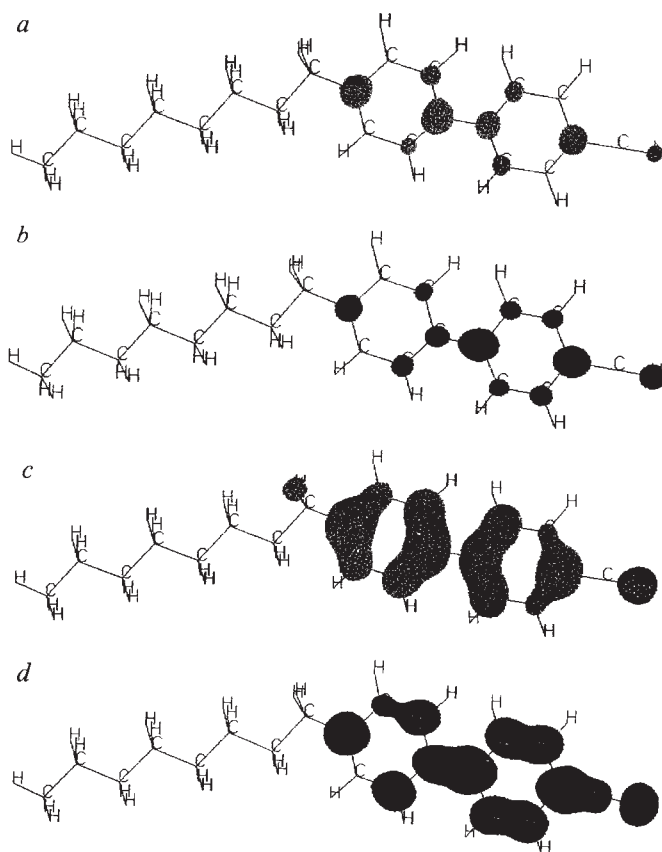


FIG. 4 Molecular orbital calculations of a free 8CB molecule. *a*, HOMO calculation showing the surface whose molecular orbital density (in units of 10^{-3} (bohr radius)⁻³) corresponds to $\phi^2=8.1$. *b*, LUMO calculation for $\phi^2=8.1$. *c*, HOMO, $\phi^2=1.6$. *d*, LUMO, $\phi^2=1.6$. The results are in good agreement with the data shown in Figs 2 and 3. The fact that the LUMO and HOMO contours are very similar is consistent with the experimental observation that reversing the polarity of the tunnel bias did not significantly alter the STM images. The *ab initio* Hartree-Fock calculations were performed using the program Gaussian 86 (ref. 12) assuming that the molecular orbitals are linear combinations of the hydrogen 1s and the carbon and nitrogen 1s, 2s, 2p_x, 2p_y and 2p_z states. The calculations do not take into account modifications of the orbitals due to the proximity of the tip, substrate and neighbouring molecules. Others have successfully used such simple calculations on free molecules to interpret STM results⁹.

which senses total electron density, would not resolve a large difference between the different functional groups.

Finally, we would like to point out that the ability of alkyl chains to readily absorb onto graphite may be a useful way to pin molecules to this substrate in order to examine them with the STM. □

Received 22 December 1989; accepted 2 March 1990.

1. Foster, J. S. & Frommer, J. E. *Nature* **333**, 542-545 (1988).
2. Smith, D. P. E., Hörber, H., Gerber, Ch. & Binnig, G. *Science* **245**, 43-45 (1989).
3. Everett, D. H. & Findenegg, G. H. *Nature* **223**, 52-53 (1969).
4. Foster, J. S., Frommer, J. E. & Spong, J. K. *Proc. SPIE* **1080**, 200-208 (1989).
5. McGonigal, G. C., Bernhardt, R. H. & Thomson, D. J. *Appl. Phys. Lett.* (in the press).
6. Berker, A. N., Ostlund, S. & Putnam, F. A. *Phys. Rev. B* **17**, 3650-3665 (1978).
7. Groszek, A. J. *Proc. R. Soc. Lond. A* **314**, 473-498 (1970).
8. Hansma, P. K. & Tersoff, J. *J. appl. Phys.* **61**, R1-R23 (1987).
9. Lippel, P. H., Wilson, R. J., Miller, M. D., Wöll, Ch. & Chiang, S. *Phys. Rev. Lett.* **62**, 171-174 (1989).
10. Spong, J. K. *et al. Nature* **338**, 137-139 (1989).
11. Hansma, P. K., Elings, V. B., Marti, O. & Bracker, C. E. *Science* **242**, 209-242 (1988).
12. Frisch, M. J. *et al. Gaussian 86* (Carnegie-Mellon Quantum Chemistry Publishing Unit, Pittsburgh, 1984).

ACKNOWLEDGEMENTS. We thank N. Honjou for assisting with the molecular orbital calculations, M. Niksch for developing the image processing software, and T. W. Hänsch and J. E. Frommer for helpful discussions.

Changes in the global concentration of tropospheric ozone due to human activities

Adrian M. Hough & Richard G. Derwent

Modelling and Assessments Group, Environmental and Medical Sciences Division, Harwell Laboratory, Didcot, Oxfordshire OX11 0RA, UK

THERE is evidence from records of ground-level measurements¹⁻⁶ that the average tropospheric concentration of ozone in the Northern Hemisphere has increased. In particular, the comparison of recent observations with those made at the Montsouris laboratory in Paris between 1876 and 1910^{5,6}, suggests that the surface concentration of ozone at mid to high latitudes has more than doubled in the past 100 years. This has potential implications for a wide range of environmental issues both because of the direct effects of elevated concentrations of ozone on man and ecosystems^{7,8} and because ozone is a radiatively active gas which could contribute significantly to global warming if its concentration were to increase⁹⁻¹². We have used a global tropospheric model to simulate the chemistry of the pre-industrial atmosphere and that of the present day. The model results for surface ozone concentrations in the pre-industrial atmosphere agree well with the Montsouris data, and the calculated concentrations for the present day agree with recent observations of a wide range of chemical species. Estimates of the future growth in emissions of nitrogen oxides (NO_x) (ref. 13) have been used to make similar calculations for the year 2020. On the basis of these estimates, the global tropospheric concentration of ozone will continue to increase at a rate faster than during the past 100 years. The potential for further increases in tropospheric ozone needs to be taken into account when assessing the impact of air pollution emissions and the adequacy of measures to control them.

In this study, a two-dimensional (latitude-altitude) model has been used to describe the global life cycles of methane, carbon monoxide, hydrogen, nitrogen oxides and 11 hydrocarbons. The model domain extends from the north to the south pole and from the surface to 24 km, and is divided into 288 equal-volume grid-cells, by a mesh with 24 horizontal divisions in sine(latitude) and 12 equally spaced vertical layers. The model describes the emissions, chemistry, transport and deposition of the major tropospheric trace gases, as shown in Fig. 1. A complete description of the model chemistry, emissions inventory and results, including a comparison with observations, is given elsewhere¹⁴. The model is built upon a basic understanding of the emissions and atmospheric chemistry and is insensitive to the initial concentrations apart from that for methane. This is because most species have lifetimes of less than one month, whereas the model simulations were run for several years. For methane, the initial concentrations were based on observations¹⁵ and on measurements of methane in ice-cores^{16,17}. All two-dimensional models share the limitation that they cannot resolve east/west or land/sea differences at a given latitude. This limitation must be remembered when interpreting model results. In the future, three-dimensional modelling studies should remove this difficulty.

Figure 2a shows the monthly variation in ozone concentrations for 48–56° N both for the pre-industrial atmosphere and for the present day. There is good agreement between model and observations for the present day, whilst the results of the pre-industrial model are a little higher than the Montsouris observations. This discrepancy may indicate that model emissions of NO_x are a little too large in the pre-industrial scenario. Alternatively, the two-dimensional model could be underestimating destruction of ozone by reaction with natural NMHC (non-methane hydrocarbons) over land. Both model and observations indicate that, on average, the surface ozone

concentration has more than doubled in the past 100 years.

Figure 3b shows the increase in model ozone between the pre-industrial atmosphere and the present day during the month of April. The actual model results for the pre-industrial atmosphere are shown in Fig. 3a. The large increase at high northern latitudes is clearly visible, being in excess of 40 parts per 10^9 (by volume). This corresponds to the region of highest surface NO_x emissions. The smallest change occurs in the tropics, due to the short lifetime of NO_x in this region, and the strong vertical transport that is present. Comparison of the model results with those from a model that ignores chemical processes suggests that the pre-industrial lower troposphere was a net sink for ozone, whereas at present it acts as a net source. The increase in surface ozone registered by the difference between the Montsouris and present-day observations^{5,6} (see Fig. 2a) is therefore likely to be a sign of a larger-scale increase throughout the whole of the troposphere. The calculations show that there is a plausible explanation for this increase in ozone, based on current understanding of how man may have influenced the life cycles of the major tropospheric trace gases: NO_x , CH_4 and CO .

Two scenarios for the year 2020 were also constructed, using the estimated bounds on NO_x emissions of Galloway¹³, who derived his values from projected population changes¹⁸ and limits on the energy production per head of population (see caption to Fig. 1). His upper bound for NO_x is similar to values obtained by projecting the rates of increase in NO_x emissions from developing nations between 1970 and 1980 forward to the year 2020, using the data of Dignon and Hameed¹⁹, whilst holding emissions from the developed nations constant. It is also consistent with the increase in energy consumption for the same regions derived by the World Energy Conference²⁰. By these estimates, over 65% of NO_x emissions in 2020 could

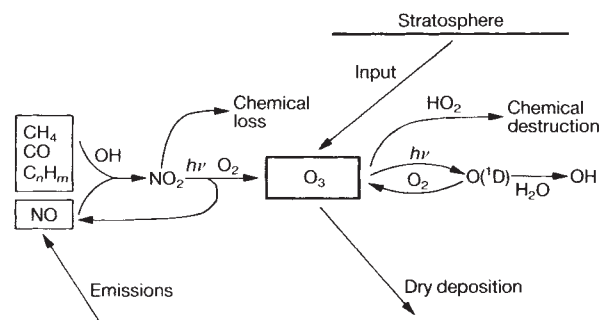


FIG. 1 The principal processes affecting the atmospheric life cycles of methane (CH_4), carbon monoxide (CO), hydrocarbons (C_nH_m) and ozone (O_3), as represented in the model. The atmospheric circulation used is that of Plumb and Mahlmann²⁴, and the method used to calculate the photolysis rates and the upper boundary condition for ozone have been described in detail elsewhere^{25,26}. The emissions rates were derived from published data (for example, refs 27–30 and references therein) and were prescribed as detailed functions of latitude and season. The total emissions of each of the principal species in MT yr^{-1} summed over all sources including natural emissions were: methane: 264; 595; 742; 767; carbon monoxide: 285; 1,575; 2,100; 3,075; hydrocarbons: 102; 220; 274; 319; NO_x (as N): 14.6; 42; 53; 87, where the figures are for pre-industrial, 1980s, 2020 (lower bound) and 2020 (upper bound) scenarios respectively. The figures for hydrocarbons exclude emissions of isoprene and terpenes, and the figures for NO_x include 8 MT from lightning. The changes in the emissions between the 1980s and 2020 are consistent with the changes in population and energy use used by Galloway in projecting upper and lower bounds on industrial NO_x emissions for 2020¹³. His lower bound was calculated by assuming that the commercial energy consumption per head of population will remain constant, and by taking account of the projected increase in population for each continent¹⁴. The upper bound was calculated for the same projected change in population, but by assuming that the rate of increase in energy consumption per head of population in the developing countries will be the same as between 1956 and 1978.

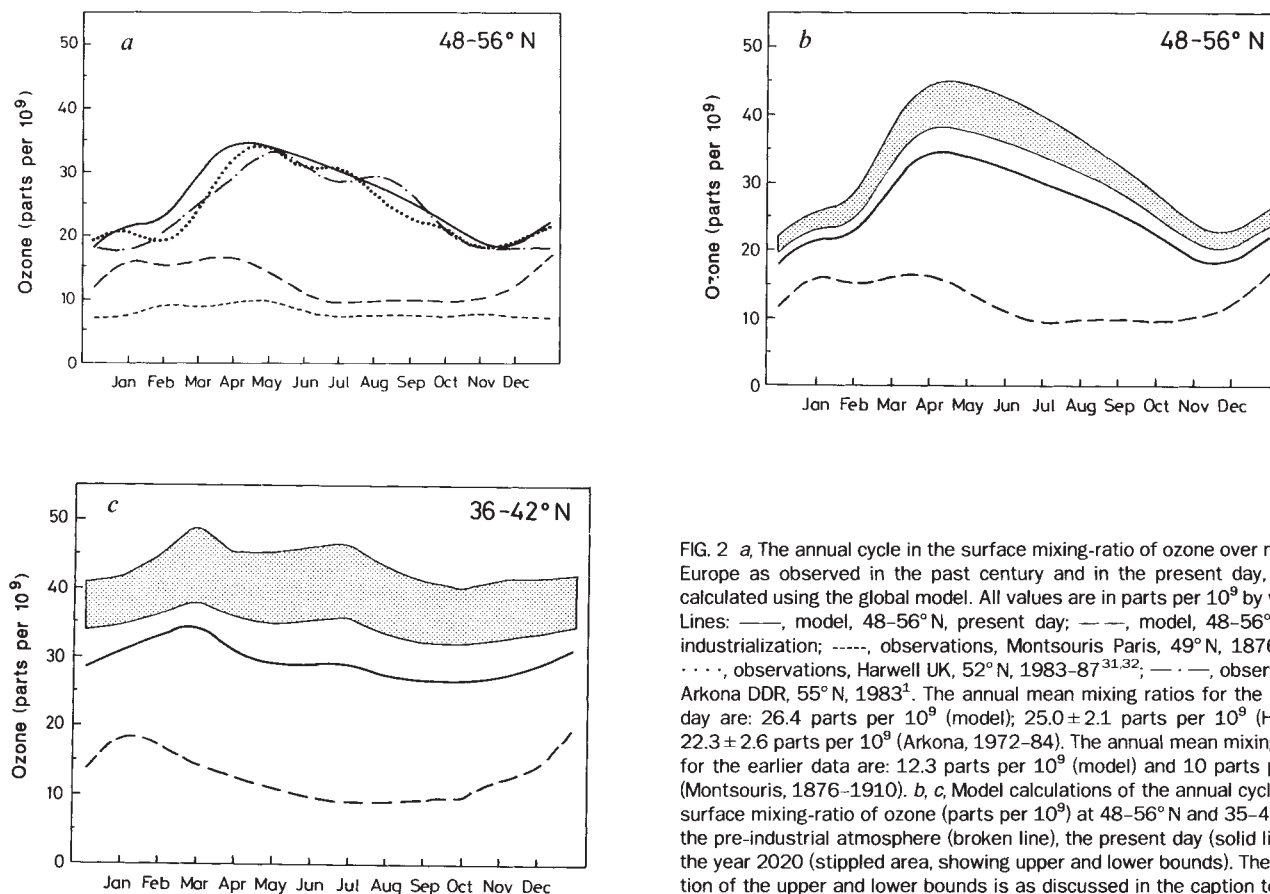


FIG. 2 *a*, The annual cycle in the surface mixing-ratio of ozone over northern Europe as observed in the past century and in the present day, and as calculated using the global model. All values are in parts per 10^9 by volume. Lines: —, model, 48–56° N, present day; — —, model, 48–56° N, pre-industrialization; ----, observations, Montsouris Paris, 49° N, 1876–86^{5,6}; ····, observations, Harwell UK, 52° N, 1983–87^{31,32}; — · —, observations, Arkona DDR, 55° N, 1983¹. The annual mean mixing ratios for the present day are: 26.4 parts per 10^9 (model); 25.0 ± 2.1 parts per 10^9 (Harwell); 22.3 ± 2.6 parts per 10^9 (Arkona, 1972–84). The annual mean mixing ratios for the earlier data are: 12.3 parts per 10^9 (model) and 10 parts per 10^9 (Montsouris, 1876–1910). *b*, *c*, Model calculations of the annual cycle in the surface mixing-ratio of ozone (parts per 10^9) at 48–56° N and 35–41° N for the pre-industrial atmosphere (broken line), the present day (solid line) and the year 2020 (stippled area, showing upper and lower bounds). The derivation of the upper and lower bounds is as discussed in the caption to Fig. 1.

TABLE 1 Ozone budgets (MT yr^{-1}) for the four model scenarios

Budget terms	Pre-industrial	Model scenarios		
		1980s	2020 (lower)	2020 (upper)
Sources:				
Stratospheric processes*	1,220	1,220	1,220	1,220
HO ₂ + NO → NO ₂ †	1,550	3,360	4,080	5,390
CH ₃ O ₂ + NO → NO ₂ †	550	1,200	1,510	1,760
RO ₂ + NO → NO ₂ †	340	570	670	930
Sinks:				
Dry deposition	740	1,420	1,660	2,075
O(¹ D) + H ₂ O†	1,440	2,260	2,580	3,050
O(³ P) loss‡	25	25	25	25
O ₃ + NO → NO ₂ §	50	140	180	300
O ₃ + NO ₂ → NO ₃	5	30	45	90
O ₃ + hydrocarbons	150	175	190	210
O ₃ + HO ₂ and OH	1,250	2,300	2,800	3,550
Total source or sink	3,660	6,350	7,480	9,300
Inventory (MT) in January	910	995	1030	1085
Average lifetime of ozone (days)	91	57	50	43
Non-stratospheric production	2,440	5,130	6,260	8,080
Non-stratospheric production 30–90° N	345	1,380	1,700	2,300
Production at 30-90° N as % of global	14.1	26.9	27.2	28.5

All figures are in MT yr^{-1} ($10^{12} \text{ g yr}^{-1}$) and have been rounded to the nearest 5 MT ($5 \times 10^{12} \text{ g}$) unless otherwise indicated.

* Transport of ozone into the model domain from above, plus chemical production by the stratospheric chemical processes: $\text{O}_2 \xrightarrow{\text{h}\nu} 2\text{O}(^3\text{P})$ followed by $\text{O}(^3\text{P}) + \text{O}_2 \rightarrow \text{O}_3$.

† Oxidation of NO to NO_2 followed by the chemical processes: $\text{NO}_2 \xrightarrow{\text{h}\nu} \text{NO} + \text{O}(^3\text{P})$; $\text{O}(^3\text{P}) + \text{O}_2 \rightarrow \text{O}_3$. RO_2 is the sum of all organic peroxy radicals excluding CH_3O_2 . HO_2 is formed primarily in the oxidation of CO, which is both emitted at the surface of the Earth and formed in the oxidation of methane and other hydrocarbons.

‡ Loss of oxygen atoms formed in the photolysis of ozone. (The majority of such oxygen atoms react with oxygen molecules to reform ozone.)

§ Oxidation of NO to NO_2 by ozone, where the NO_2 is not subsequently photolysed to oxygen atoms.

|| Oxidation of NO_2 to NO_3 by ozone, where the NO_3 does not subsequently yield an oxygen atom.

originate in developing nations. The industrial emissions of hydrocarbons and carbon monoxide were increased by the same factor as NO_x . The initial mixing-ratio for methane was set 600 parts per 10^9 greater than the present day, consistent both with estimated source strengths and mixing rates in the twenty-first century¹¹ and with the present rate of increase^{16,17,21}.

Figure 2b shows the seasonal variation in surface ozone from 48–56° N (latitudes typical of northern Europe and southern Canada) for pre-industrial, present-day and 2020 scenarios. It is clear that there is the potential for a substantial increase in the ozone mixing ratio, and that the spring maximum is becoming more pronounced. Figure 2c shows similar information for latitudes typical of central USA, Japan and China. Here the seasonal variation is much smaller, and the potential increase in ozone is greater than at more northerly latitudes. These values represent the average background concentration upon which local- and regional-scale photochemical formation of ozone in the boundary layer will be superimposed. Therefore, this possible background increase could reduce the apparent impact of ozone control measures on long-term average ozone concentrations.

Figure 3c shows the change in tropospheric ozone between the present day and the upper estimate for 2020, during April. Compared with Fig. 3b the maximum increase is not quite as large and is further south, reflecting the change in the distribution of the emissions. Through the tropics and Southern Hemisphere, as well as in the upper troposphere, the corresponding change is greater than in Fig. 3b. Overall, on the basis of the present assumptions, the increase in the concentration of tropospheric ozone during the next 30 years could be equivalent to that already experienced over the past 100 years.

The calculated sources and sinks for ozone, summed over the whole model domain, are shown in Table 1. There is an almost constant baseline contribution of $\sim 1,220 \text{ MT yr}^{-1}$ from stratospheric processes, with the increase between the different

scenarios being caused by additional oxidation of NO to NO_2 with its subsequent photolysis to $\text{O}(^3\text{P})$ atoms which react with O_2 to produce O_3 . Some 65% of this oxidation is performed by the HO_2 radical which is itself formed in the photochemical degradation of carbon monoxide, methane and other hydrocarbons. The majority of the remaining oxidation is performed by the CH_3O_2 radical, which is formed primarily from the degradation of methane, the atmospheric concentration of which is increasing^{16,17,21}. Given the present assumptions, the overall non-stratospheric production of ozone has doubled between pre-industrial times and the present day, and has increased by a factor of four in the region 30–90° N. There has therefore been a marked shift in the distribution of ozone production in the lower atmosphere.

Whether any of these statements concerning past, present and future ozone concentrations in the troposphere reflect what has happened or is likely to happen in the real atmosphere depends on the adequacy and completeness of the model used and that of its input data. Model evaluations of the global production and loss of ozone are limited by current understanding of the budget and distribution of tropospheric NO_x (ref. 22). Two-dimensional models imply averaging around latitude circles, that is, over spatial scales that are considerably larger than the mean transport distances of NO_x . Under these circumstances, coarse-resolution two-dimensional models may overestimate photochemical ozone production and underestimate photochemical ozone destruction. Box model studies²³ have shown the importance of nonlinear chemistry on tropospheric ozone production and how this nonlinearity is influenced by the representation of the combination reactions of peroxy radicals, night-time NO_x loss and non-methane hydrocarbon chemistry. These aspects have been given a fully and explicit treatment in our chemical scheme and this should serve to minimize errors in the influence of nonlinear chemistry on the two-dimensional ozone budget calculations. Nevertheless, the concern about

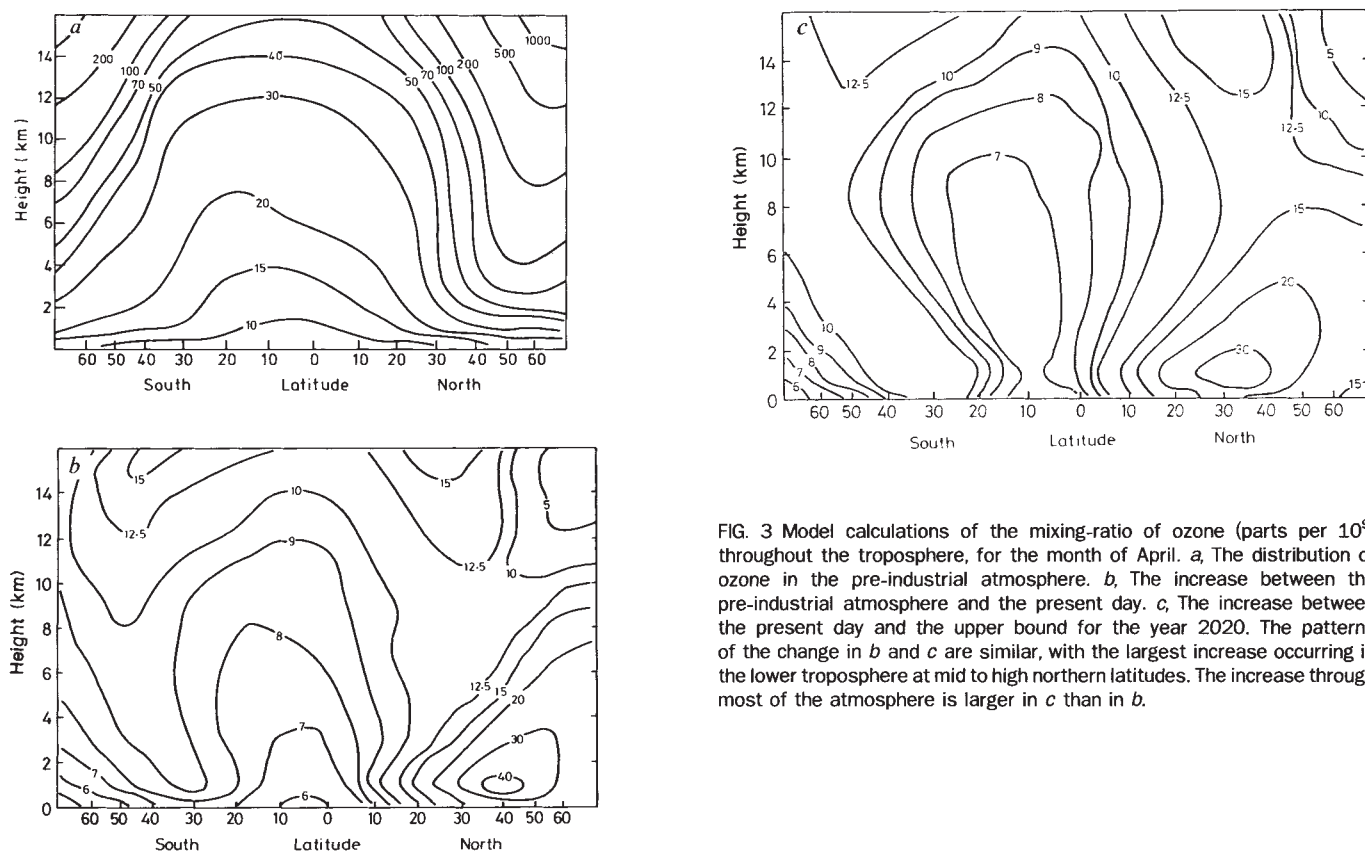


FIG. 3 Model calculations of the mixing-ratio of ozone (parts per 10^9) throughout the troposphere, for the month of April. a, The distribution of ozone in the pre-industrial atmosphere. b, The increase between the pre-industrial atmosphere and the present day. c, The increase between the present day and the upper bound for the year 2020. The patterns of the change in b and c are similar, with the largest increase occurring in the lower troposphere at mid to high northern latitudes. The increase through most of the atmosphere is larger in c than in b.

spatial averaging in two-dimensional models still remains and this can be removed only by the application of three-dimensional modelling techniques.

The model illustrates the potential for further large increases in the concentration of tropospheric ozone on a global scale. Furthermore, the largest increase is likely to occur at mid to high latitudes in the Northern Hemisphere, the region where the largest increases have occurred in the past, despite a shift of industrial and urban development towards lower latitudes. These increases are driven by increasing emissions of nitrogen oxides, compounded by rising concentrations of methane and other hydrocarbons.

The overall capacity for tropospheric ozone production is very large. The limited realization of this potential in the pre-industrial atmosphere is shown by the model to be due to the limited availability of NO_x . The modelled increase in ozone is caused by the increased availability of NO_x , and to some extent methane and carbon monoxide, due to human activity, and is consistent with the measured increase in the Northern Hemisphere. The potential for further increases in ozone should be taken into account when assessing the impact of air-pollution emissions and the adequacy of measures to control them. Future work will need to address the current limited understanding of the global budget and distribution of NO_x . □

Received 9 May 1989; accepted 20 February 1990.

- Feister, U. & Warmbt, W. *J. Atmos. Chem.* **5**, 1–21 (1987).
- Oltmans, S. J. & Komhyr, W. D. *J. geophys. Res.* **91**, 5229–5236 (1986).
- Bokkov, R. D. *Proc. Adv. Res. Workshop on Tropospheric Ozone*, Lillehammer, Norway, June 1–5, 1987 (ed. Isaksen, I.S.A.), 83–96 (Reidel, Dordrecht, 1987).
- Logan, J. A. *J. geophys. Res.* **90**, 10,463–10,482 (1985).
- Volz, A. & Kley, D. *Nature* **332**, 240–242 (1988).
- Kley, D., Volz, A. & Mülheims, F. *Proc. Adv. Res. Workshop on Tropospheric Ozone*, Lillehammer, Norway, June 1–5, 1987 (ed. Isaksen, I.S.A.), 63–72 (Reidel, Dordrecht, 1987).
- Tillon, B. E. *Environ. Sci. Tech.* **23**, 257–263 (1989).
- Guderian, R., Becker, K. & Schurath, U. *Ecological Studies* **52** (Springer-Verlag, Berlin, 1984).
- Ramanathan, V., Cicerone, R. J., Singh, H. B. & Kiehl, J. T. *J. geophys. Res.* **90**, 5547–5566 (1985).
- Dickinson, R. E. & Cicerone, R. J. *Nature* **319**, 109–115 (1986).
- Bolle, H.-J., Seiler, W. & Bolin, B. *The greenhouse effect, climatic change and ecosystems. SCOPE 29* (ed. Bolin, B., Döös, B. R., Jäger, J. & Warrick, R. A.) 157–203 (Wiley, New York, 1986).
- Ramanathan, V. *The changing atmosphere, Dahlem Workshop Rep.* (ed. Rowland, F. S. & Isaksen, I. S. A.) 159–186 (Wiley, New York, 1988).
- Galloway, J. N. *Ambio* **18**, 161–166 (1989).
- Hough, A. M. *The development of a two-dimensional global tropospheric model: the model chemistry* (Harwell Laboratory, Didcot, 1990).
- Steele, L. P. *et al. J. Atmos. Chem.* **5**, 125–171 (1987).
- Rasmussen, R. A. & Khalil, M. A. K. *J. geophys. Res.* **89**, 11,599–11,605 (1984).
- Khalil, M. A. K. & Rasmussen, R. A. *Atmos. Environ.* **21**, 2445–2452 (1987).
- United Nations Secretariat. *Population Bulletin of the United Nations, No. 14* (United Nations, New York, 1983).
- Dignon, J. & Hameed, S. *J. Air Pollut. Control Assoc.* **39**, 180–186 (1989).
- World Energy Conference. *Energy 2000–2020, World Prospects and Regional Stresses* (Graham and Trotman, London, 1983).
- Blake, D. R. & Rowland, F. S. *J. Atmos. Chem.* **4**, 43–62 (1986).
- Liu, S. C. *et al. J. geophys. Res.* **92**, 4191–4207 (1987).
- Lin, X., Trainer, M. & Liu, S. C. *J. geophys. Res.* **93**, 15,879–15,888 (1987).
- Plumb, A. & Mahlmann, J. D. *J. Atmos. Sci.* **44**, 298–327 (1987).
- Hough, A. M. *The calculation of photolysis rates for use in global tropospheric modelling studies* AERE R-13259 (HMSO, London, 1989).
- Hough, A. M. & Woods, K. J. *Ozone concentrations in the global atmosphere* AERE R-13271 (HMSO, London, 1988).
- Isaksen, I.S.A. & Hov, O. *Tellus* **39B**, 271–285 (1987).
- Bingemer, H.G. & Crutzen, P. J. *J. geophys. Res.* **92**, 2181–2187 (1987).
- Matthews, E. & Fung, I. *Global Biogeochemical Cycles* **1**, 61–86 (1987).
- Warneck, P. *The Chemistry of the Natural Atmosphere* (Academic Press, San Diego, 1988).
- Sandalls, F. J. & Gaudern, S. L. *Measurements of ozone, nitric oxide and nitrogen dioxide in ambient air at Harwell, Jan–Dec 1986* AERE R-12560 (HMSO, London, 1987).
- Sandalls, F. J., Graves, M. J. & Gaudern, S. L. *Measurements of ozone, nitric oxide and nitrogen dioxide in ambient air at Harwell, Jan–Dec 1987* AERE R-13076 (HMSO, London, 1988).

ACKNOWLEDGEMENTS. This work was funded by the United Kingdom Department of the Environment, as part of its air pollution research programme.

Characterization of unresolved complex mixtures of hydrocarbons in petroleum

M. A. Gough* & S. J. Rowland

Petroleum and Environmental Geochemistry Group,
Department of Environmental Sciences, Polytechnic South West,
Drake Circus, Plymouth, PL4 8AA, UK

ALTHOUGH petroleum is an important energy resource, there is still much to learn about its composition that would be of practical importance in exploration, refining and the identification of materials resulting from oil spills. Chromatography does not resolve (and thus identify) a substantial proportion of even the hydrocarbons in petroleum. These components are often referred to as the unresolved complex mixture (UCM), or 'hump', which is especially pronounced for biodegraded petroleum and certain refined fractions such as lubricating oils^{1–4}. We now describe the use of several methods in parallel to analyse the hydrocarbon hump from crudes, lubricating oils and oil-spill samples. Comparison of our results with those for synthesized alkanes allows us to propose model structures for some of the hydrocarbon components of the hump, and from *in vitro* studies we conclude that the resistance of many of these compounds to microbial degradation arises partly from their structure.

Aliphatic hydrocarbon UCMs were isolated from a variety of petroleum fractions by a combination of column and thin-layer chromatographic methods (including argentation TLC), and triplicate urea adductions (Table 1). These were then

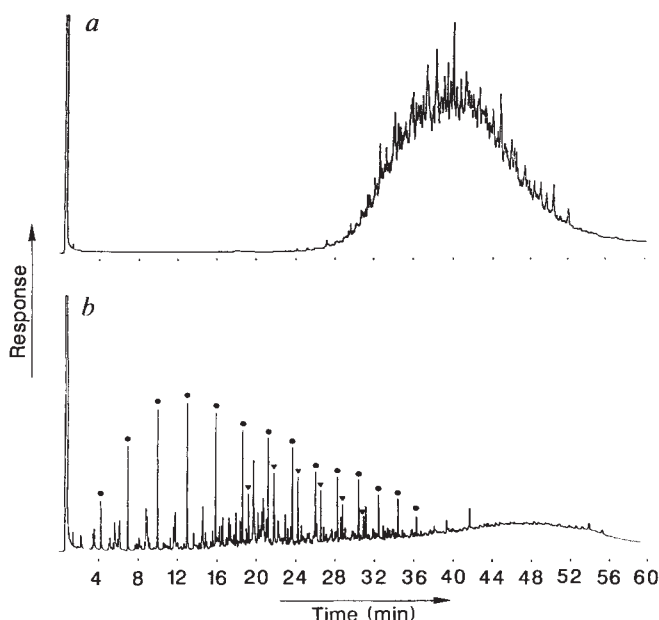


FIG. 1 a, UCM derived from column chromatography (hexane eluate), argentation TLC and urea adduction (urea non-adduct) of a lubricating base oil (Silkolene 150). GC: 25-m fused silica column DB-5 (J&W Scientific). Resolved components (<10%) identified as mainly acyclic isoprenoid alkanes (C_{24} – C_{35}). b, Products obtained by chemical oxidation with chromium trioxide. n -acids (●), C_6 – C_{19} , n - α , ω -diacids (▼), C_8 – C_{13} , γ -methyl- γ -lactones and methyl ketones.

* Present address: Marine Organic Chemistry Group, Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth, PL1 3DH, UK.

TABLE 1 Isolation and oxidation of hydrocarbon UCMs

Oil	Alkanes* (%)	UCM† (%)	Total yield (%)	Oxidized‡ yield (%)
Silkolene 150	80 ± 3§	91 ± 3§	80	71
Shell paraffinic lubricating	69	83	75	73
Shell naphthenic lubricating	66	94	73	69
Nigerian crudes (<i>Sivand</i> cargo)	55	76	83	—
Humber estuary (<i>Sivand</i> spill)	43	87	83	—
Amoco tank oil	16	68	87	—
Newgale beach (Amoco tank spill)	16	21	75	—
Athabasca oil sand, bitumen	8	46	93	—

* Obtained by column and silver ion TLC.

† Obtained by urea adduction (non-adduct = UCM) expressed as a proportion of total alkane fraction.

‡ Oxidized material refers to that fraction not eluting with hexane from a silica gel chromatography column and includes mono- and di-carboxylic acids, ketones and lactones (see text).

§ Average of four analyses.

|| Average of two analyses.

screened by high-temperature gas chromatography (GC) (up to C_{70}), and selected samples examined by elemental analysis, gel permeation chromatography, field ionization-mass spectrometry (FI-MS), probe electron impact-mass spectrometry (EI-MS) and chemical ionization-mass spectrometry (CI-MS)⁵. Carbon type analysis (refractive index/kinematic viscosity: nd/V_k) data was also available for two of the lubricating oils.

Samples were carefully selected to include the hydrocarbon base stocks of various well characterized lubricating oils, two well documented environmental oil spills, and a widely reported heavily biodegraded petroleum. The proportion of non-aromatic UCM in all was high, which facilitated the isolation of milligram quantities. The spectral and chromatographic data of the paraffinic lubricating base oils were consistent with a high proportion of acyclic and monocyclic alkanes, where as expected, the naphthenic lubricating oil comprised a higher proportion of cyclic hydrocarbons.

Chromium trioxide oxidation of each UCM isolate using a modification of an earlier procedure⁶ produced variable proportions of products. Chromatographic fractionation of total recovered material showed that the proportion of oxidized material was high (>71%; Table 1). Non-recovered material was assumed to be lost as gas because water-soluble products were absent⁵.

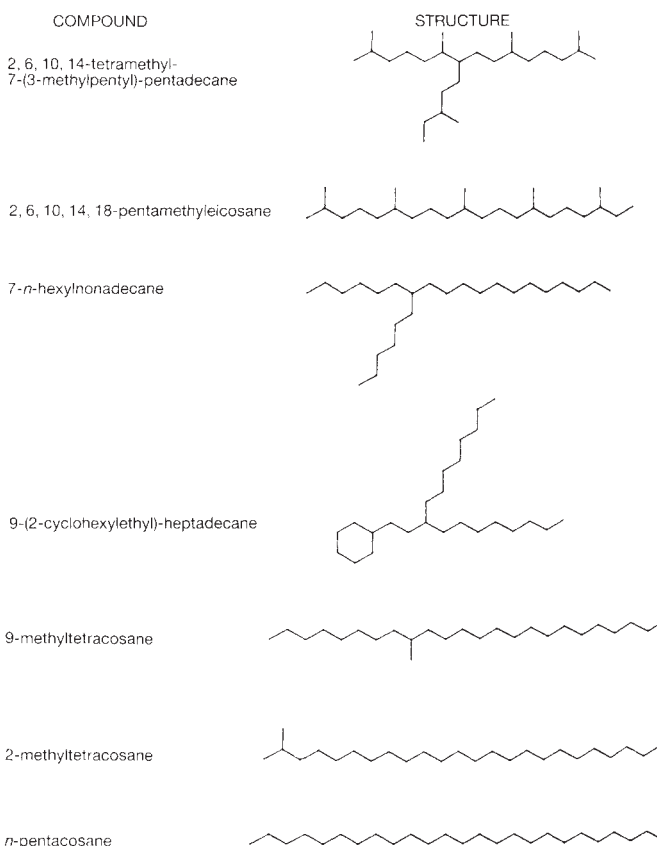
Perhaps most interestingly, the oxidation of the UCM hydrocarbons resulted in the formation of many products which were resolved by capillary GC (Fig. 1). These were identified by EI and CI GC-MS and by comparisons with synthesized compounds, as homologous series of normal and branched monocarboxylic acids, α,ω -dicarboxylic acids, γ -lactones and ketones. In all cases the major resolved components were straight-chain monocarboxylic acids, with cyclic products present only in minor quantities. The latter result was somewhat surprising as previous authors have often assumed the UCM to comprise highly branched and/or cyclic hydrocarbons, and may be partly due to ring opening during oxidation. Possible precursors of the straight-chain products include simple monoalkyl substituted T-branched alkanes (see Fig. 2). According to the reported mechanism of CrO_3 oxidations, these would cleave preferentially at the tertiary centre after hydroxylation and dehydration⁷. Given a carbon number range between C_{20} and C_{30} , there exist 536 possible T-branched acyclic alkanes of this type, a mixture which in itself would probably be difficult to resolve by capillary GC.

If the UCMs do consist in part of such simple compounds, it is perhaps surprising that they show such resistance to biodegradation^{2,8,9}. To investigate this, we synthesized six C_{25}

hydrocarbons ranging from monosubstituted to hexa-substituted acyclic, and a branched alkyl-substituted monocyclic hydrocarbon (Fig. 2). Results of *in vitro* biodegradation of these compounds and of a hydrocarbon lubricating base oil (Silkolene 150) UCM with a pure culture of *Pseudomonas fluorescens* for 25 days showed that the model T-branched alkanes were resistant relative to the normal and monomethyl-branched compounds. Indeed, their rate of degradation was similar to that of the UCM and to the highly branched acyclic isoprenoids. These results support our hypothesis that UCMs contain, in part, monoalkyl-substituted T branched alkanes.

Oxidation of the synthetic alkanes under identical conditions to the UCMs confirmed the mechanism in ref. 7 (that is, production of *n*-acids). Recoveries of oxidation products suggested that only the acyclic isoprenoids were underestimated.

Although oxidation of each of the UCMs produced similar resolved products, the proportions of these relative to each other and to residual or functionalized UCM varied. In fact, the distributions of the minor components were, somewhat surprisingly, sufficiently different to allow each UCM to be distinguished by GC-MS. This is demonstrated by application to residues from two oil spills. In September 1983 ~6,000 tonnes of Nigerian crude oils were spilt into the Humber Estuary, UK, from the tanker *Sivand*². The oil became rapidly associated with surficial sediments and with time was subjected to microbial degradation and other weathering processes. Isolation and oxidation of urea non-adducted (mainly UCM) hydrocarbons from the original cargo oils, and from an estuarine mud sample collected one year after the spill, produced similar resolved distributions of acids, ketones and lactones (Fig. 3). Similarly, the oxidation products of urea non-adducted hydrocarbons derived from an oil that had leaked from a ruptured refinery oil tank (Milford Haven, UK) were similar to those obtained by oxidation of a residue that came ashore 10 days after the

FIG. 2 Synthetic model C_{25} hydrocarbons.

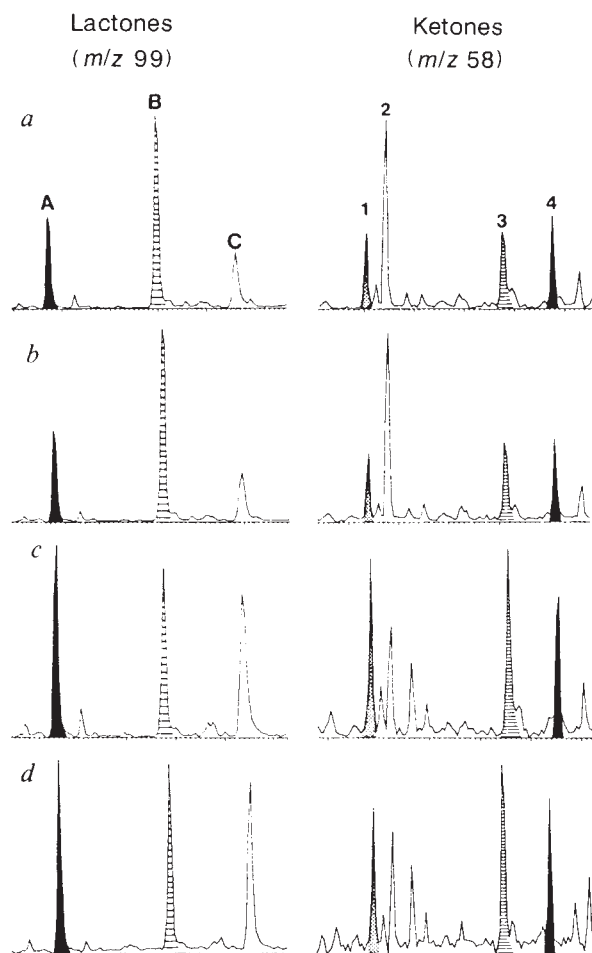


FIG. 3 Comparative partial GC-MS mass fragmentograms of γ -methyl- γ -lactones ($m/z = 99$) and alkyl ketones ($m/z = 58$) observed for each of the products of oxidation of aliphatic UCMs. Compounds A and C are C_{11} and C_{12} γ -methyl- γ -lactones, whereas compound B is tentatively assigned as a C_{11} methyl-branched γ -methyl- γ -lactone. Compounds 1 and 4 are straight-chain C_{12} and C_{13} methyl ketones and are derived from methyl-branched compounds in the UCM. Compounds 2 (6,10-dimethylundecan-2-one) and 3 (8-oxo-4-methylnonanoic acid) are obtained from acyclic isoprenoid alkyl moieties. a, Sivand cargo oil; b, Humber Estuary sediment; c, Amoco tank oil; d, Newgate Beach sediment.

spill¹⁰. Although these urea non-adducted hydrocarbons also contained some resolved isoprenoid compounds before oxidation (for instance pristane, steranes, triterpanes and other biological marker compounds commonly used in oil fingerprinting studies¹¹), the distribution of products after oxidation in each of these cases provided a useful additional diagnostic fingerprint of the more abundant UCM hydrocarbons. It remains to be seen whether this is more generally applicable.

These differences in UCM composition probably reflect differences in the kerogen from which the oils, including the UCMs, are derived. Our initial experiments suggest that UCMs can be produced from polymethylene polymers by laboratory maturation, perhaps as a result of chain scission and recombination to produce mixtures of T-branched alkanes. □

Received 14 December 1989; accepted 26 January 1990.

- Blumer, M., Ehrhardt, H. & Jones, J. H. *Deep Sea Res.* **20**, 239–259 (1973).
- Jones, D. M., Rowland, S. J., Douglas, A. G. & Howells, S. *Int. J. environ. analyt. Chem.* **24**, 227–247 (1986).
- Rubinstein, I., Strausz, O. P., Spyckerelle, C., Crawford, R. J. & Westlake, D. W. S. *Geochim. cosmochim. Acta* **41**, 1341–1353 (1977).

- Tanacredi, J. T. *J. Water Poll. Control Fed.* **2**, 216–226 (1977).
- Gough, M. A. thesis, Polytechnic South West UK (1989).
- Brooks, P. W., Maxwell, J. R., Cornforth, J. W., Butlin, A. G. & Milne, C. B. in *Advances in Organic Geochemistry 1975* (eds Campos, R. & Goni, J. J. 81–97 (Enadisma, Madrid, 1977).
- Canelli, G. & Cardillo, G. *Chromium Oxidations in Organic Chemistry* (Springer-Verlag, Berlin, 1984).
- Volkman, J. K., Alexander, R., Kagi, R. I. & Woodhouse, G. W. *Geochim. cosmochim. Acta* **47**, 785–794 (1983).
- Volkman, J. K., Alexander, R., Kagi, R. I., Rowland, S. J. & Sheppard, P. N. *Org. Geochem.* **6**, 619–632 (1984).
- Howells, S. E., Patey, J., Turner, C. & Dodd, N. M., Oil Pollution Research Unit Rep. No. 27 (Field Studies Council, Angle, Dyfed, Wales, 1986).
- Albaiges, J. & Albrecht, P. *Int. J. environ. analyt. Chem.* **6**, 13–15 (1979).

ACKNOWLEDGEMENTS. We thank A. Revill for access to unpublished data, R. Srodzinski for help with GC-MS and S. Hird for assistance with figures. We also thank Silkstone UK, Shell UK, M. Fowler and S. Howells for provision of samples, and A. Aldridge for assistance with computing. We thank M. Rhead for valuable discussions.

Evidence for a smaller magma chamber beneath the East Pacific Rise at 9°30' N

G. M. Kent, A. J. Harding & J. A. Orcutt

Institute of Geophysics and Planetary Physics, Scripps Institution of Oceanography, UCSD, La Jolla, California 92093, USA

THE size and shape of magma chambers beneath mid-ocean ridges are fundamental features that control the availability of melt, composition of magmas and formation of oceanic crust. Models derived from the study of ophiolites¹, and from thermal considerations², include a very large magma chamber, which can exceed 10–20 km in width. Cross-axis seismic reflection profiles from the East Pacific Rise, however, constrain the width of the axial magma chamber to be <3–4 km (ref. 3). Even this may be an over-estimate, arising from the under-migration of diffracted energy generated at the edges of a smaller magma chamber. Here we show that forward modelling of these diffraction hyperbolae yields a distance between the best-fitting point diffractors, and by inference a magma chamber width, of only 800–1,200 m. Reflectivity modelling also suggests that the available data are consistent with a magma chamber comprising only a thin layer of melt. A narrow and thin axial magma chamber would inhibit along-axis mixing⁴, and might thereby account for variations in magma composition along the East Pacific Rise⁵.

The East Pacific Rise at 9°30' N is characterized as a fast-spreading centre (55–60 mm yr⁻¹ half rate)⁶ and its broad topographic signature has been associated with a robust magma supply⁷. The presence and dimensions of the magma chamber, and its corresponding region of suppressed seismic velocities, have been estimated using ocean-bottom seismometer^{8,9} and multichannel seismic techniques^{3,10–13}. Recent interpretations of expanding-spread-profile (ESP) lines reveal a low-velocity zone extending at least 6–7 km in width and centred about the rise-axis^{12,13}. Conventional common depth point (CDP) studies have shown the axial magma chamber (AMC) reflector to be generally 2–3 km in width on the migrated sections³. The AMC reflector has been interpreted as originating from the roof of the magma chamber^{3,10,11}, whereas the low-velocity zone may represent a broad zone of hot rock containing a small percentage of partial melt^{12–14}. If these physical models are correct, the width and thickness of the magma chamber obtained from reflection profiles can give important constraints on the total volume of magma available for an eruption.

Two CDP lines, located near 9°30' N on the East Pacific Rise, were reprocessed to obtain a better constraint on magma-chamber width. Stacking velocities were estimated from constant-velocity stacks which were calculated for every other 10 CDPs in the region of the rise-axis. If no coherent events were seen, stacking velocities were calculated using velocity-depth profiles constructed from ESP data¹². After determining

the proper stacking velocity, normal moveout corrections were applied and the data were then stacked and bandpass filtered. The AMC reflector is apparent on both stacked sections and lies ~ 0.6 s beneath the sea floor at the rise axis. Diffractions generated from the edges of the magma chamber can be seen emanating from both sides of the AMC reflector (Fig. 1a,b). The stacked sections were migrated using a finite-difference (45° approximation) algorithm^{15,16}. We chose a finite-difference scheme for our migration algorithm because of its ability to incorporate smoothly varying velocity fields, thus enabling a first-order correction for varying water depth in our wavefield extrapolation. The apparent migrated widths of the AMC reflector on CDP lines 29 and 31 were 1,575 m and 1,925 m, respectively.

An alternative method for calculating magma-chamber width is to forward model the diffractions seen on the stacked section by ray-tracing. We have constructed a velocity model of the upper oceanic crust using SeaBeam bathymetry and a velocity-depth function calculated from an ESP on the rise axis¹². Every twenty-fifth centre-beam depth (~ 400 m spacing) was used to construct the sea floor from which the velocity-depth function was hung (that is, the velocity contours are assumed to follow the topography). Point diffractors were placed in the velocity mesh with their lateral positions constrained by the migrated image, and their depths obtained from the velocity-depth function. Rays were traced analytically from each point diffractor to receivers on the surface with a travel time calculated for each path. Placing arrival times beneath the corresponding receivers,

a synthetic diffraction curve was thereby modelled on a zero-offset section. For diffracted energy, a stacked section is a good approximation to the zero-offset case, thus allowing synthetic diffraction curves to be plotted on our stacked profiles.

Using the migrated width to constrain the lateral positions of the point diffractors, we were unable to match successfully the diffraction patterns on the stacked section; however, by moving the point diffractors closer together in our model, a satisfactory fit was obtained. The best-fitting widths for CDP lines 29 and 31 were 800 m and 1,200 m, respectively (Fig. 1c,d). The discrepancy between migrated and best-fitting widths may be wholly, or in part, due to the following. (1) A finite depth-step ($\tau = 0.025$ s) in our finite-differencing scheme inherently produces an under-migration of the image. (2) Axial topography reduces stacking velocities which can result in reduced estimates of migration velocities. (3) The finite-difference migration algorithm assumes that the velocity is locally constant, and therefore is unable to account for refraction effects at interfaces (for example, the water/sea-floor interface). Because of the inherent problems with migration, and the excellent velocity control in the upper crust, we believe that the widths calculated using synthetic diffractions are more reliable.

The presence of magma-chamber diffractions on the stacked sections requires a sharp contrast in acoustic impedance for their generation. This contrast probably originates from an abrupt termination of melt at the edges of the magma chamber. If this hypothesis is true, estimates in width using point diffractor analysis place strict limits on the lateral extent of the magma

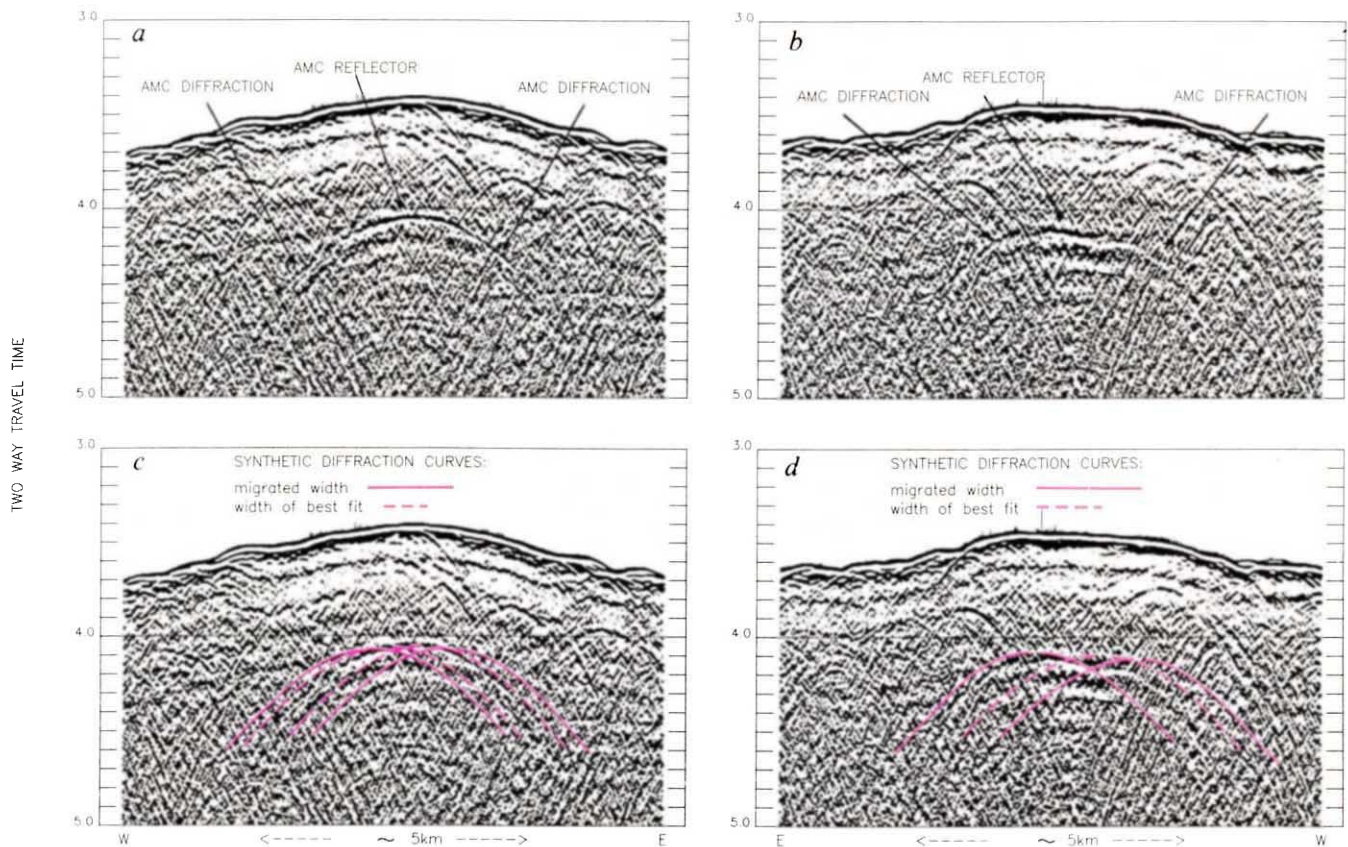


FIG. 1 Common-mid-point stacks. *a*, CDP line 29 and *b*, CDP line 31 which are located at $9^\circ 37' \text{ N}$ and $9^\circ 30' \text{ N}$, respectively. The magma-chamber diffractions can be seen emanating from the AMC reflector which lies ~ 0.6 s beneath the sea floor at the rise-crest. Synthetic diffraction curves are plotted on stacked sections *c*, CDP 29 and *d*, CDP 31. Solid lines were generated from points diffractors whose lateral positions were constrained from the migrated image to be (*c*) 1,575 m and (*d*) 1,925 m apart. Dashed

lines were produced from point diffractors whose lateral positions best fitted the data and were (*c*) 800 m and (*d*) 1,200 m apart. The solid and dashed diffraction curves for the left point diffractor in (*d*) are overlain, thus concealing the dashed curve for the best-fitting point diffractor. The solid and dashed lines in the uppermost portion of (*c*) and (*d*) reflect the position and estimated width of the magma chamber.

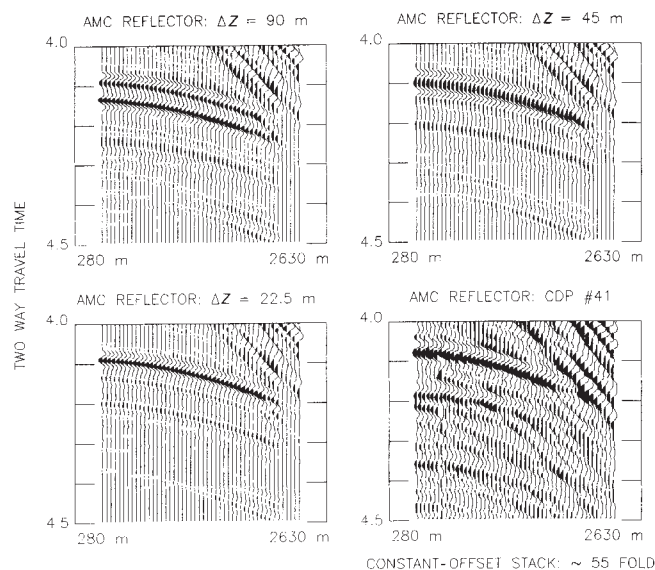


FIG. 2 Reflectivity synthetics calculated with varying thicknesses of melt (ΔZ) are compared to a constant-offset stack of along-axis data (CDP 41). The source-time function used in our reflectivity calculations was derived from a near-offset stack of along-axis data. The bubble-pulse was included in our source-time function and can be seen ~ 0.1 s after the initial reflection. The magma-chamber reflection seen in the constant-offset stack and synthetics is phase reversed with respect to the sea-floor reflection. Interference effects mask the presence of the basal reflector for thicknesses ≤ 50 m (reflections from the top and bottom of the magma chamber can be seen for $\Delta Z = 90$ m).

chamber. Many proposed models for the magma chamber are capable of producing diffractions seen in the data^{13,17,18}, but these models include dipping sides and/or bottom which have not been observed to date. A model that satisfies both diffraction and basal reflector requirements is a thin lens of buoyant magma trapped at the top of the low-velocity zone. In this model, interference effects between the top and bottom reflectors mask the presence of the magma-chamber bottom.

To test our thin-lens hypothesis, we used the reflectivity method to model the response of a layered medium¹⁹. We used an ESP velocity model¹² of the upper-oceanic crust, which included a thin layer of melt at its base, in our calculation of

synthetics. The acoustic impedance contrast across the upper and lower boundaries of the melt lens was assumed to be equivalent, thus representing a worst-case model for concealing the basal reflection. The thickness of melt was then varied to enable the mapping of AMC reflector variability versus layer thickness. To facilitate comparison of reflectivity models with real data, we stacked constant-offset traces along the rise-axis in the region of the two cross-axis lines interpreted above. Along-axis data were chosen to minimize topographic effects and increase the multiplicity of the data, and hence the signal-to-noise ratio.

Results from reflectivity modelling reveal interference effects between the wavelet reflecting off the top (phase reversed) and bottom (not phased reversed) of a magma lens as its thickness decreases. Comparison between a constant-offset stack of along-axis data and synthetics show that a magma chamber comprising a thin layer of melt (~ 10 – 50 m) is consistent with the available data (Fig. 2). To check the consistency of our model, it was necessary to calculate synthetics for large offsets and compare them to ESP data¹²; no discrepancies were found. Alternatively, the absence of an observed basal reflection could be the result of a gradual increase of velocities across the lower boundary (for example, a transition from mesh to mush) resulting in a reduced acoustic impedance contrast. The possibility of a lower-boundary gradient model existing precludes any strict upper bounds on the magma lens thickness; however, a thin lens of melt (~ 10 – 50 m) is consistent with the available seismic data, and therefore, a potential model for the magma chamber.

An important consideration with the melt lens model (Fig. 3) is whether a thin layer of melt could be the sole source of extrusives generated at the East Pacific Rise. Assuming an eruption interval of 50–600 yr²⁰ and a spreading rate of 55–60 mm yr⁻¹ (ref. 6), the volume of extrusives produced during an eruption is nearly equivalent to the volume of melt within our model; therefore, it is unnecessary to require alternative sources of melt to explain the generation of extrusives along the rise-crest. If our melt lens model of the magma chamber is correct, then the volume of melt beneath the rise-axis is much less than previously inferred. Such a model would be in direct contradiction to thermal models which require a very large magma chamber^{2,18,21}. Refraction studies^{12–14} and gravity modelling²², however, do not require a magma chamber of any appreciable size and are consistent with a thin layer of melt. We believe that our model may also explain the variability of the AMC reflector on the along-axis line. The AMC reflector has been described as continuous along-axis³, but the reflector has many gaps, even in regions where topography would suggest

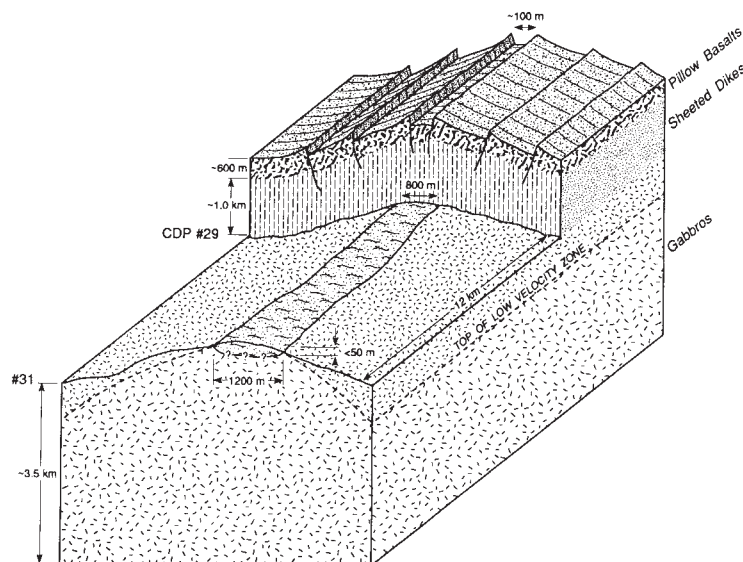


FIG. 3 A schematic cross-section of the proposed melt lens model for the magma chamber at 9°30'N. The extrusives have been partially stripped away to show the along-axis variations in magma-chamber width (800–1,200 m). The suggested thickness for the melt layer is ~ 10 – 50 m; however, question marks at the base of this layer indicate the uncertainties of our result. The axial summit graben, which has been correlated with the presence of a magma chamber⁷, is ~ 100 m wide in this region. The low-velocity zone imaged from ESPs^{12,13} originates at the top of the magma chamber and shows significant deepening relative to the sheeted dikes/gabbro interface. The reduction of seismic velocities in this region is thought to result from hot rock containing a small percentage of partial melt^{12–14}.

a continuous magma chamber⁷. If the magma chamber is a thin layer of melt, it is more plausible to expect gaps in the image of the reflector resulting from interference effects as the thickness of the layer decreases to zero (or resulting from navigational error which no longer places the ship above the narrow body); however, if the magma chamber is very large, it becomes difficult to explain the discontinuous nature of the reflector since this would imply a closely spaced and segmented system of large magma chambers along the rise-crest. A thin layer might also inhibit along-axis mixing of chemically distinct magmas⁴, and therefore, help explain geochemical discontinuities which are not correlated with any tectonic features⁵. This model may challenge the view that these bodies of melt are steady-state features in both time and space—they may be quite transient features of a dynamic ridge system. □

Received 27 November 1989; accepted 20 February 1990.

- Pallister, J. S. & Hopson, C. A. *J. geophys. Res.* **86**, 2595–2644 (1981).
- Sleep, N. H. *J. geophys. Res.* **80**, 4037–4042 (1975).
- Detrick, R. S. *et al. Nature* **326**, 35–41 (1987).
- Oldenburg, C. M., Spera, F. J., Yuen, D. A. & Sewell, G. *J. geophys. Res.* **94**, 9215–9236 (1989).
- Langmuir, C. H., Bender, J. F. & Batiza, R. *Nature* **322**, 422–429 (1986).
- Klitgord, K. D. & Marmmerickx, J. *J. geophys. Res.* **87**, 6725–6750 (1982).
- Macdonald, K. C. *et al. Nature* **335**, 217–225 (1988).
- Rosendahl, B. R. *et al. J. geophys. Res.* **81**, 5294–5304 (1976).
- Orcutt, J. A., Kennett, B. L. N., Dorman, L. & Prothero, W. *Nature* **256**, 475–476 (1975).
- Herron, T. J., Stoffa, P. & Buhl, P. *Geophys. Res. Lett.* **7**, 989–992 (1980).
- Hale, L. D., Morton, C. J. & Sleep, N. H. *J. geophys. Res.* **87**, 7707–7719 (1982).
- Vera, E. E. *et al. J. geophys. Res.*, submitted (1989).
- Harding, A. J. *et al. J. geophys. Res.* **94**, 12163–12196 (1989).
- Burnett, M. S., Caress, D. W. & Orcutt, J. A. *Nature* **339**, 208–220 (1989).
- Claerbout, J. F. *Fundamentals of Geophysical Data Processing* (McGraw-Hill Book Co., Inc., New York, 1983).
- Brysk, H. *Geophysics* **48**, 532–542 (1983).
- McClain, J. S., Orcutt, J. A. & Burnett, M. S. *J. geophys. Res.* **90**, 8627–8639 (1985).
- Morton, J. L. & Sleep, N. M. *J. geophys. Res.* **90**, 11345–11353 (1985).
- Kennett, B. L. *Seismic Wave Propagation in a Stratified Media* (Cambridge University Press, New York, 1983).
- Macdonald, K. C. *et al. Rev. Earth planet. Sci.* **10**, 155–190 (1982).
- Wilson, D. S., Clague, D. A., Sleep, N. H. & Morton, J. L. *J. geophys. Res.* **93**, 11974–11984 (1988).
- Madsen, J. A., Detrick, R. S., Mutter, J. C., Buhl, P. & Orcutt, J. A. *J. geophys. Res.*, submitted (1989).

Volatile-rich mantle fluids inferred from inclusions in diamond and mantle xenoliths

G. Turner*, R. Burgess* & M. Bannont†

* Department of Geology, University of Manchester, Manchester M13 9PL, UK

† Department of Physics, University of Sheffield, Sheffield S3 7RH, UK

THE ^{40}Ar – ^{39}Ar technique has been used to unscramble mixtures of radiogenic, parentless (excess) and atmospheric argon components in (hydrothermal) fluid inclusions through correlations with K, Cl and ^{36}Ar respectively¹. These procedures are also applicable to mantle-derived argon isotopes in diamond², previously reported as having anomalously high (6 Gyr) K–Ar ages³. Here we describe ^{40}Ar – ^{39}Ar measurements on coats and cores of ‘coated stones’ similar to those analysed for trace elements by Navon *et al.*⁴, and on fluid inclusions in olivine from an East African mantle xenolith with mid-ocean-ridge basalt (MORB) affinities⁵. Our results prove conclusively that ^{40}Ar in the coats of the diamonds, and in the mantle xenolith, is present in a widespread chlorine-rich component. These data, in conjunction with those of Navon *et al.*⁴, imply the existence of $\text{H}_2\text{O}/\text{CO}_2$ -rich phases with $^{40}\text{Ar}/\text{Cl}$ ratios which are remarkably uniform over large distances, and which show enrichments of these two incompatible elements by almost four orders of magnitude relative to bulk upper-mantle values. These extreme enrichments imply that the fluid phase must be present at no more than one or two parts in 10^4 in the region from which the argon and chlorine are extracted.

We have analysed 12 fragments of inclusion-bearing coats and six fragments of inclusion-free cores from four Zaire ‘coated stones’. The bulk chemical data are summarized in Table 1. Detailed results will be published elsewhere. The average sample weight of 3.4 mg in Table 1 is a factor of 70 smaller than that used by Ozima *et al.*². Nevertheless our system blanks are sufficiently small for us to perform more detailed stepped heating experiments in which argon was released in 12 heating steps between 500 °C and 2,150 °C rather than the four high-temperature steps in the previous study. Two major release peaks were observed for all isotopes. The pattern shown in Fig. 1 is based on the ^{38}Ar release from coat 3B and is typical of all the samples analysed. The major release at 2,000 °C corresponds to graphitization. The release at 1,400 °C has not been observed previously in diamond and may be the result of decrepitation of inclusions, or an effect associated with reactor-induced radiation damage, or both. Detailed experiments on unirradiated samples may clarify this point. Ozima *et al.*² speculated that noble gases released in the single heating step which they employed below the graphitization temperature, were of lower-mantle origin and contrasted with those of upper-mantle origin released on graphitization. Our more detailed experiment indicated no major compositional distinction between argon released in the high- and low-temperature peaks. Low ($^{40}\text{Ar}/^{36}\text{Ar}$) ratios associated with low amounts of gas release do not require an exotic explanation and can be most easily understood in terms of the (atmospheric) argon blanks.

In contrast to the earlier studies (refs 2 and 3), the rough correlation between ^{40}Ar and K breaks down completely when a greater number of heating steps is used. Plotted on the conventional ‘isochron diagram’ (Fig. 2a), the data from stones 1–3 scatter widely and do not define a unique age. In contrast the data form a well defined linear array on a plot of ($^{40}\text{Ar}/^{36}\text{Ar}$) against ($\text{Cl}/^{36}\text{Ar}$) (Fig. 2b). This array is interpreted as a mixing line between a Cl-correlated excess ^{40}Ar component and a Cl-free component. The intercept of the graph indicates that ($^{40}\text{Ar}/^{36}\text{Ar}$) for the Cl-free component is 320 ± 60 . This is, within error, equal to the atmospheric ratio and as argued above is most simply understood as adsorbed atmospheric argon blank. The slope of the graph is $(7.8 \pm 0.2) \times 10^{-4}$ M and is indistinguishable from the value of $(8.4 \pm 0.7) \times 10^{-4}$ observed by the

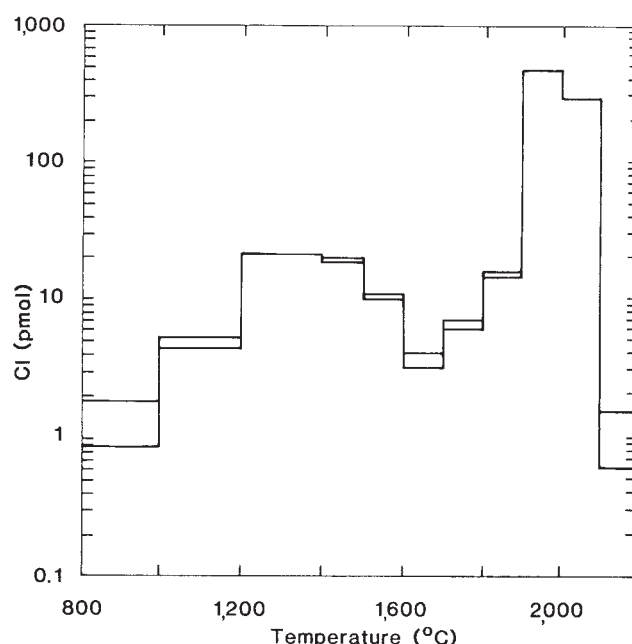


FIG. 1 Example of a stepped heating release pattern for ^{38}Ar , produced by neutron activation of Cl, expressed as equivalent amount of Cl (pmol).

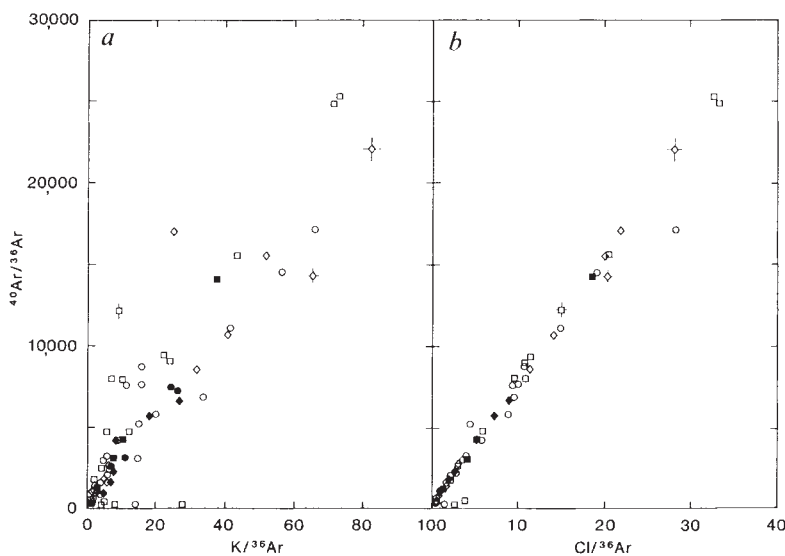


FIG. 2 $^{40}\text{Ar}/^{36}\text{Ar}$ against $\text{K}/^{36}\text{Ar}$ ('isochron' diagram) and against $\text{Cl}/^{36}\text{Ar}$. The absence of a correlation between ^{40}Ar and K and the high $^{40}\text{Ar}/\text{K}$ ratios indicate that the ^{40}Ar is not produced by *in situ* radioactive decay. Stone 1, circles; stone 2, squares; stone 3, diamonds; open symbols are temperature steps, and solid symbols are bulk composition obtained by summing temperature steps. All ratios are molar.

Tokyo group² on four unrelated Zaire stones. Stone 4 shows a similarly good correlation but with a significantly higher slope ($13 \pm 1 \times 10^{-4}$). Navon *et al.*⁴ analysed this stone (their sample Z4) and found it to be unusual.

Maximum ($^{40}\text{Ar}/^{36}\text{Ar}$) ratios for stones 1–3 range from 17,000 to 25,000 and thus have a clear modern upper-mantle signature⁶. Stone 4 has a maximum ($^{40}\text{Ar}/^{36}\text{Ar}$) ratio of 40,000 which exceeds any (MORB) mantle ratio so far observed and possibly reflects differences between oceanic and subcontinental mantle, that is, a higher time-integrated ($\text{K}/^{36}\text{Ar}$) ratio for the sub-continental mantle. The very low ($\text{K}/^{40}\text{Ar}$) ratios (implicit in the >6 Gyr apparent ages) indicate that the ($^{40}\text{Ar}/^{36}\text{Ar}$) ratio in the inclusions has not changed significantly since they formed. Models of mantle evolution indicate that ($^{40}\text{Ar}/^{36}\text{Ar}$) has increased throughout Earth history and thus the observation of a ratio in diamond which is close to the modern mantle value provides a semi-quantitative argument that the volatile enrichment and the formation of the inclusions occurred relatively recently. Maximum ($\text{Cl}/^{36}\text{Ar}$) ratios are similar in all four samples and may also be typical of the upper mantle. It is interesting to note that they are roughly two to three times the value for sea water.

A small correction for the presumed atmospheric ^{40}Ar (based on $296 \times ^{36}\text{Ar}$) has been applied to the data shown in Fig. 3, in which ($^{40}\text{Ar}^*/\text{K}$) is plotted against (Cl/K). The tentative correlation observed by Ozima *et al.* is clearly confirmed, largely

because of the much wider (by a factor of six) range in (Cl/K), 0.13–1.65, and in ($^{40}\text{Ar}^*/\text{K}$), 0.25×10^{-4} to 13.5×10^{-4} . The range in the (meaningless) apparent K–Ar age is also wider, 2.0–8.5 Gyr. We suspect that some of the variation in (Cl/K) may be an artefact resulting from recoil of ^{39}Ar (ref. 7) from the K-rich micro-inclusions to the K-poor diamond matrix; we plan to investigate this possibility further. The bulk compositions cannot be affected by recoil and the factor-of-two variation in (Cl/K) and ($^{40}\text{Ar}^*/\text{Cl}$) in Fig. 3 (solid symbols) is therefore real and represents either a variation in the composition of the mantle fluids or a chemical fractionation occurring as the inclusions form.

The Cl- and Ar-rich phase was found in all but one of the cores (Table 1) at concentrations roughly 1/5 those of the corresponding coats. This is surprising given that on visual inspection and on the basis of infrared spectra (H. J. Milledge, personal communication) the coats seemed to be free of micro-inclusions. A possible explanation is the presence of coat material introduced to the core by way of cracks (O. Navon, personal communication). The ($^{40}\text{Ar}^*/\text{Cl}$) ratio for core 1 is significantly higher than the coat, possibly reflecting more than one generation of inclusions.

We have also done combined crushing and stepped heating experiments on a mantle olivine sample which contains fluid inclusions. This olivine is from a phlogopite-amphibolite vein in a spinel lherzolite xenolith in a recent volcanic (Pello Hill

TABLE 1

Sample	Weight (mg)	Cl (p.p.m.)	K (p.p.m.)	$^{36}\text{Ar}^\dagger$ ($10^{-9} \text{ cm}^3 \text{ g}$)	^{40}Ar	$^{40}\text{Ar}^*/\text{Cl}$ (10^{-6} M)
Zaire 1 coat‡	4.1	7.5	20.4	0.6	3,420	670
core	3.3	1.1	3.2	0.3	780	940
Zaire 2 coat‡	6.4	13.8	29.8	1.2	6,990	760
core B	1.3	4.9	10.8	2.3	3,030	760
core C	5.5	3.5	7.5	0.6	1,820	750
Zaire 3 coat ‡	3.2	8.4	22.7	1.0	4,290	760
core A	2.7	3.2	11.3	1.1	1,740	700
core B	3.3	0.2	0.6	0.3	170	—
Zaire 4 coat‡	4.4	17.3	65.2	1.1	15,210	1,370
core	7.4	2.7	11.1	0.4	2,210	1,250
Mantle olivine§	94.6	0.46	4.9	0.79	350	400

† No blank correction applied, see text.

‡ Average of 3, 2, 4 and 3 samples for coats 1, 2, 3 and 4 respectively.

§ Concentrations from crushing only.

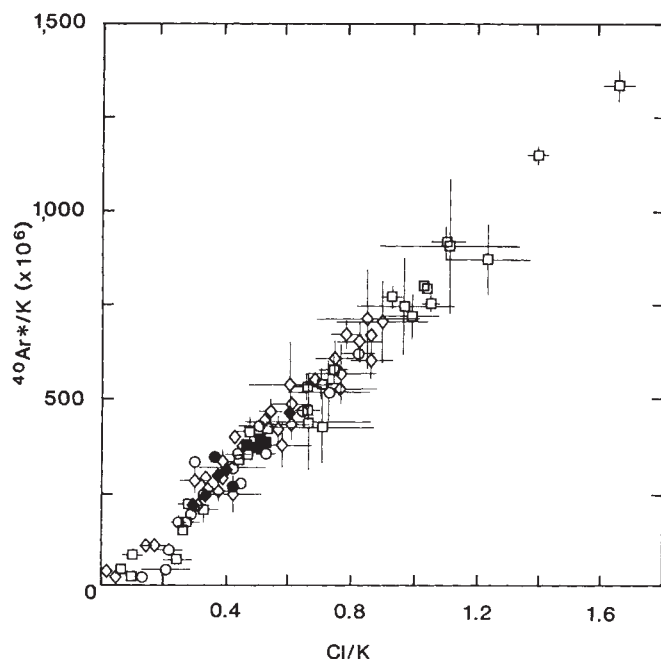


FIG. 3 $^{40}\text{Ar}^*/\text{K}$ against Cl/K . $^{40}\text{Ar}^*$ is the ^{40}Ar remaining after applying a standard correction for a presumed modern atmospheric (blank) component, based on ^{36}Ar . The correlation is interpreted as a mixing line between fluids with a roughly constant ($^{40}\text{Ar}/\text{Cl}$) ratio and variable (Cl/K). Some of the apparent variation in (Cl/K) may result from ^{39}Ar recoil but the bulk compositions (solid symbols) indicate that at least a factor-of-two variation is real. Symbols as Fig. 2. Ratios are molar.

tuff) from Tanzania. The isotope geochemistry of both vein and host have been described by Cohen *et al.*⁵. They concluded that whereas the xenolith had Sr, Nd and Pb isotope systematics indicative of 2.0-Gyr-old subcontinental lithosphere, the vein was recent and had the characteristics of depleted (MORB-type) mantle. The stepped heating results are not reported here beyond stating that the K-Ar systematics are consistent with the recent eruption age. The results of the crushing experiments, which release gas from the fluid inclusions, are more interesting and are summarized in Table 1.

The average ($^{40}\text{Ar}/^{36}\text{Ar}$) ratio of the argon released by crushing is low (450) and indicates the presence of modern atmosphere in some of the inclusions. The olivine contains both primary inclusions, distributed throughout the mineral, and secondary inclusions in later rehealed fractures. We assume that the latter are recent and contain modern meteoric water, with ($^{40}\text{Ar}/^{36}\text{Ar}$) = 296. If this atmospheric component is subtracted in the normal way the ($^{40}\text{Ar}^*/\text{Cl}$) ratio calculated is 4×10^{-4} . This ratio is similar to the ratio reported above for the fluid in diamonds and is much higher than ratios observed in inclusions in crustal rocks¹. We suppose that it relates to argon and chlorine in primary inclusions. The actual ratio in the primary inclusions could be higher (but not lower) than 4×10^{-4} , if some of the Cl is associated with the 'meteoric' component. Whatever the exact value, the similarity to the diamond values is striking and provides further evidence for a strong coherence between the behaviour of Ar and Cl in upper-mantle fluids. The observation

is also consistent with the interpretation of Cohen *et al.*⁵ that the vein represents depleted (MORB-type) upper mantle.

The uniformity of the ($^{40}\text{Ar}/\text{Cl}$) ratio in the seven of the eight diamonds analysed so far indicates either that the mantle fluid incorporated in them is widespread and well mixed on the scale of the diamond source region, or that fluids have quantitatively extracted the highly incompatible ^{40}Ar and Cl from a source region which is itself very uniform. If, as the xenolith result suggests, the ratio is typical of the upper mantle, it can be used to estimate a Cl abundance based on existing estimates of upper-mantle ^{40}Ar abundance (or vice versa). For example, a Cl abundance of 8 p.p.m. is calculated based on $^{40}\text{Ar} = 4 \times 10^{-6} \text{ cm}^3 \text{ STP g}^{-1}$ (corresponding to 85% outgassing of ^{40}Ar from the upper mantle)⁸. The abundance of Cl in the mantle is not well established. Based on abundances in MORB glasses and model calculations, Schilling *et al.*⁹ estimate a value of 7 p.p.m. for the depleted upper-mantle source region.

Ion-probe measurements by Navon *et al.*⁴ indicate K abundances between 7 and 16 wt% in the inclusions when H_2O and CO_2 are included. Combined with our average K/Cl ratio of around 3 this indicates that Cl is present in the inclusions at between 2 and 5 wt% and ^{40}Ar at an astonishing $1\text{--}3 \times 10^{-2} \text{ cm}^3 \text{ STP g}^{-1}$. These concentrations correspond to enrichments by almost four orders of magnitude over upper-mantle values. To achieve this level of enrichment the $\text{H}_2\text{O}/\text{CO}_2$ -rich fluid phase must be present in the region from which the Cl and Ar are extracted at a level of less than one or two parts in 10^4 . □

Received 25 September 1989; accepted 20 February 1990.

1. Turner, G. *Geochim. cosmochim. Acta* **52**, 1443–1448 (1988).
2. Ozima, M., Zashu, S., Takigami, Y. & Turner, G. *Nature* **337**, 226–229 (1989).
3. Zashu, S., Ozima, M. & Nitoh, O. *Nature* **323**, 710–712 (1986).
4. Navon, O., Hutcheon, I. D., Rossman, G. R. & Wasserburg, G. J. *Nature* **335**, 784–789 (1988).
5. Cohen, R. S., O'Nions, R. K. & Dawson, J. B. *Earth planet. Sci. Lett.* **68**, 209–220 (1984).
6. Sarda, P., Staudacher, Th. & Allegre, C. J. *Earth planet. Sci. Lett.* **72**, 357–375 (1985).

7. Turner, G. & Cadogan, P. H. *Geochim. cosmochim. Acta Suppl.* **5**, 1601–1615 (1974).
8. Turner, G. *J. geol. Soc. Lond.* **146**, 147–154 (1989).
9. Schilling, J.-G., Bergeron, M. B. & Evans, R. *Phil. Trans. R. Soc. Lond. A* **297**, 147–178 (1980).

ACKNOWLEDGEMENTS. We are grateful to C. T. Pillinger for providing us with an exceptionally interesting suite of samples, to M. Seal of D. Drukker & Zn. for cutting and separating coats and cores and to B. Dawson for the olivine sample. We also thank O. Navon for a helpful review of our original manuscript.

Chloroplast DNA sequence from a Miocene *Magnolia* species

Edward M. Golenberg*, David E. Giannasi†, Michael T. Clegg*, Charles J. Smiley‡, Mary Durbin*, David Henderson* & Gerard Zurawski§

* Department of Botany and Plant Sciences, University of California, Riverside, California 92521, USA

† Department of Botany, University of Georgia, Athens, Georgia 30602, USA

‡ Department of Geology and Geological Engineering, University of Idaho, Moscow, Idaho 83843, USA

§ DNAX Research Institute, Palo Alto, California 94304-1104, USA

DNA has been successfully extracted from several samples of preserved tissue, the oldest so far reported originating from a 13,000-year-old ground sloth¹. But severe damage to the preserved DNA, primarily due to oxidation of the pyrimidines¹, has prevented the acquisition of sequence data from ancient samples except in a few cases²⁻⁴. We report here the extraction of DNA from fossil leaf samples from the Miocene Clarkia deposit (17–20 Myr old), the amplification of an 820-base pair (bp) DNA fragment from the chloroplast gene *rbcL* from a fossil of the genus *Magnolia*, and its subsequent sequencing. The sequence was verified by comparison with published and unpublished *rbcL* sequences. These results extend our ability to analyse ancient DNA and may open new avenues into problems in palaeobotany, biogeography, and in the calibration of mutation rates.

The Clarkia fossil beds of northern Idaho are best represented by a 9-m thick lacustrine section of soft, finely laminated, unoxidized clays with commonly occurring interbeds of airfall volcanic ash. The Miocene lake was abruptly formed by lava-damming of an ancient stream valley. Palaeontological, taphonomic and sedimentological evidence indicate that the Miocene climate of the region was warm-temperate and humid. Sediments and fossils seem to have accumulated in an anoxic lake bottom environment; they have remained unoxidized and water-saturated to the present day⁵.

Leaves and plant reproductive organs occur in abundance and taxonomic diversity in the Clarkia flora. Most leaves are preserved as compression fossils, commonly retaining intact cellular tissue with considerable ultrastructural preservation, including cell walls, leaf phytoliths, and intracellular organelles, as well as many organic constituents such as flavonoids and steroids⁶⁻⁹. There is little evidence of post-depositional (diagenetic) change in many of the leaf fossils.

The pigmented compression fossil examined in this DNA study was classified as the fossil species *Magnolia latahensis* (Berry) Brown, a species commonly found as imprints in other Miocene flora of the Columbia Plateau region. The morphology of the leaves, and of a few gynoecia in the Clarkia flora, closely resemble extant eastern American species: *M. grandiflora* L., *M. macrophylla* Michaux, and *M. virginiana* L. In eastern North America, they are associated with the same taxa that commonly occur with the Miocene Clarkia *Magnolia*; *Taxodium*, *Nyssa*, *Persea*, *Acer*, *Liquidambar*, *Liriodendron*, *Platanus*, *Gleditsia*, *Betulaceae*, and *Juglandaceae*. Other contemporary relatives of Clarkia forest plants are now found only in eastern Asia, where they represent a climatic regime similar to that of the eastern United States. These include *Metasequoia*, *Cunninghamia*, *Glyptostrobus*, *Amentotaxus*, *Cercidiphyllum*, *Pterocarya*, various legumes, and numerous species of *Magnolia* that differ morphologically from both the eastern American species and the Miocene specimens from the Clarkia flora.

Fossil-bearing shale was exposed and the rocks carefully split to expose the compression fossils, which were immediately photographed and identified. The fossil leaf material was then

TABLE 1 Kimura three substitution type (3ST) estimates of nucleotide substitution between the fossil, *Magnolia latahensis*, *rbcL* sequence and other *rbcL* sequences based on all codon positions

Species of origin	3ST
<i>Magnolia macrophylla</i>	0.022
<i>Liriodendron tulipifera</i>	0.032
<i>Persea americana</i>	0.041
<i>Platanus racemosa</i>	0.063
<i>Fagus americana</i>	0.076
<i>Nicotiana glauca</i> ²⁵	0.080
<i>Dianthus</i> sp.	0.086
<i>Petunia hybrida</i> ²⁶	0.087
<i>Morus alba</i>	0.087
<i>Gossypium hirsutum</i>	0.095
<i>Plumbago capensis</i>	0.102
<i>Spinacea oleracea</i> ²⁷	0.103
<i>Medicago sativa</i> ²⁸	0.105
<i>Rheum</i> sp.	0.108
<i>Pisum sativum</i> ²⁹	0.110
<i>Zea mays</i> ¹¹	0.138

The *rbcL* sequences of *Liriodendron tulipifera*, *Dianthus* sp., *Gossypium hirsutum*, *Plumbago capensis*, and *Rheum* sp. were determined by D.E.G., G.Z. and M.T.C. (unpublished results). The *rbcL* sequences of *Platanus racemosa*, *Fagus americana*, and *Morus alba* were determined by E.M.G., M.T.C. and D.E.G. (unpublished results). All other un referenced sequences are given in Fig. 1.

immediately scraped into mortars and ground with dry ice to a fine powder, and the DNA extracted¹⁰.

A segment of the chloroplast gene, *rbcL*, encoding the large subunit of ribulose-1,5-bisphosphate carboxylase was isolated by selective double-stranded DNA amplification using *Taq* polymerase-mediated polymerase chain reaction (PCR). Two synthetic 30-mer primers were designed based on the *rbcL* sequence from *Zea mays* (ref. 11), from positions 234–263 (coordinate number from ATG codon) and 1,053–1,024 (c) respectively. Single-stranded DNA for sequencing was prepared in separate PCR reactions.

A double-stranded 4.2-kilobase (kb) fragment that included the *rbcL* gene was amplified from a total genomic DNA extraction of the extant *Magnolia* species *M. macrophylla*. The forward primer for the amplification was a 26-mer designed from a consensus sequence from position 41–16 (c) in the *atpB* gene of *Nicotiana tabacum* (ref. 12) and *Zea mays* (ref. 13) preserving the internal *XmaI* restriction site. The reverse primer was a 22-mer based on the *Oryza sativa* sequence from ORF 106 (ref. 14) from position 41–20 (c). (Coordinate numbers are from the ATG codons for both primers.) An *rbcL* sequence has previously been determined for *Persea americana* var. Haas from position 415 (an *EcoRI* site used in cloning) (D.H., M.D., G.Z. and M.T.C., unpublished observation). To complete the sequence information from this species, a similar amplification was carried out.

Sequencing was completed using Klenow fragment-mediated dideoxynucleotide reactions¹⁵. Sequences were obtained from both strands. In the case of the fossil *Magnolia*, both strands were sequenced twice. The *rbcL* sequences obtained from *M. macrophylla*, *M. latahensis*, and *Persea americana* are listed in Fig. 1 along with the sequence from *Liriodendron tulipifera*, a closely related species in the Magnoliaceae.

Two criteria are used to verify that the sequence derives from the fossil material. We first showed that the purported amplified fossil DNA did not arise from contamination from an outside source by comparing the sequence with other available *rbcL* sequences. We also demonstrated that the derived sequence clusters with that of extant species presumed to be related to the fossil material.

Kimura's 3ST estimates of nucleotide substitution¹⁶ were calculated on the basis of all three codon positions for the *rbcL* sequences of 16 species. The species, the source of the sequence,

METHODS. DNA was extracted from single fossil leaves scraped from rocks, by the method of Rogers and Bendich¹⁰. A second extraction buffer with SDS substituted for CTAB was also tried successfully. Reaction buffer and stock nucleotide mixes for PCR amplifications using *Taq* polymerase were made according to the manufacturer's (Perkin-Cetus) recommendation. Fifty picomoles of each primer and 2 μ l (approximately 50 ng of total nucleic acids) of the crude total genomic DNA extract from a fossil *Magnolia* sample were used for a double-stranded amplification. The reaction mix was completed to a total volume of 99.5 μ l and overlaid with 100 μ l mineral oil. The reaction mix was incubated for 2 min at 94 °C to denature potential proteases in the fossil DNA extract, and 2.5 units of *Taq* polymerase were then added. The reaction was carried out in a Cov Tempocycler for 30 cycles

consisting of 1.5 min at 94 °C, 2 min at 42 °C, and 3 min at 72 °C, followed by one extension period of 15 min at 72 °C. Separate reactions produce single-stranded DNA for sequencing. Twenty picomoles of a single primer (identical to the primers used in the original double-stranded amplification) were used per reaction. Ten microlitres of the double-stranded PCR product were used for template. Buffer composition and nucleotide concentrations were identical to those used in double-stranded amplification. The reactions were incubated at 94 °C for 2 min before the addition of *Taq* polymerase. Reaction conditions were essentially identical to the double-stranded amplification except for an annealing temperature of 48 °C. Residual primers were removed by precipitating the PCR products with a one-third volume 7.5 M ammonium acetate and one and a third volumes cold 100% ethanol³. The pellets were reconstituted in 15 µl deionized, distilled water and could be used for two or three sequencing reactions. Sequencing was completed using Klenow fragment-mediated dideoxynucleotide reactions¹². Synthetic 30-mer primers based on the *Zea mays rbcl* sequence were used to prime the sequencing reactions.

657

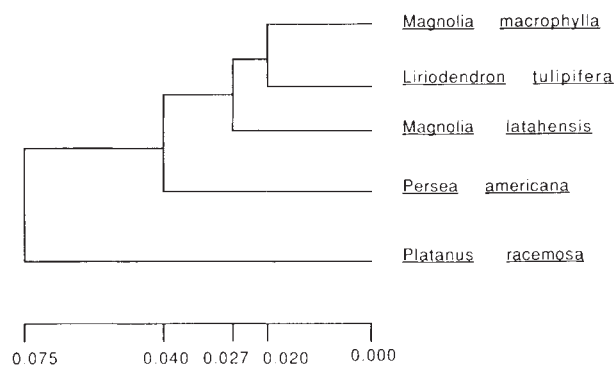


FIG. 2 Dendrogram of the clustering of the species *M. macrophylla*, *M. latahensis*, *P. americana*, *L. tulipifera*, and *P. racemosa* based on a UPGMA analysis of Kimura's 3ST estimates of nucleotide substitution based on all three codon sites in the *rbcL* sequence.

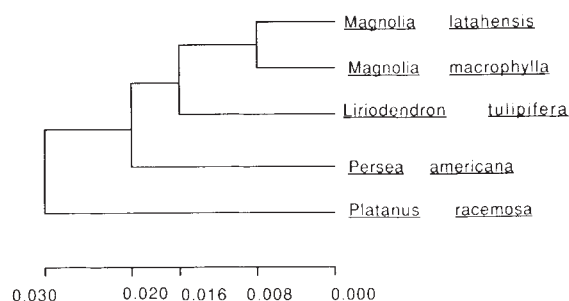


FIG. 3 Dendrogram of the clustering of the species *M. macrophylla*, *M. latahensis*, *P. americana*, *L. tulipifera*, and *P. racemosa* based on a UPGMA analysis of Kimura's 3ST estimates of nucleotide substitution based on the first and second codon sites in the *rbcL* sequence.

three species within the Magnoliaceae by this method. Thus, on the basis of its clear grouping within the Magnoliaceae and proximity to an extant *Magnolia*, we conclude that the *rbcL* sequence is from the Miocene fossil *Magnolia*.

Two factors probably enabled us to recover the fossil DNA and to amplify selectively a large segment of the *rbcL* gene. First, the chloroplast genome occurs in large numbers within plant cells¹⁹ providing a greater probability of sampling large, intact molecules. Second, the leaf material was exceptionally well preserved. Leaves had fallen nearly directly into the lake, resulting in little of the abrasion or damage characteristic of long distance transport. Modes of death and preservation of fish in the same sediments also indicate a low degree of flow disturbance, and a stable, cold hypolimnion layer, low in oxygen and rich in organic sediments²⁰. These conditions seem to have prevented extensive oxidation of pyrimidines in *Clarkia* leaves, consistent with the established preservation of the flavonoid compounds in these samples^{9,21-24}.

At an age estimated at 17–20 Myr, these samples have substantially pushed back the age of DNA that may be recovered and sequenced. This should open up possibilities of research in several new areas. First, mutation rates to outgroup species may be estimated by using the calibrated time difference between pairs of closely related extant and fossil species and their last shared ancestor. Second, sequence data and molecular phylogenetic analyses may be used independently to test purported phylogenetic relationships of extinct taxa. Third, biogeographic trends may be investigated by comparing individual relationships of species within fossil assemblages to those of existing plant communities. □

Received 1 December 1989; accepted 26 February 1990.

1. Paabo, S. *Proc. natn. Acad. Sci. U.S.A.* **86**, 1939–1943 (1989).
2. Paabo, S. *Nature* **314**, 644–645 (1985).
3. Paabo, S., Gifford, J. A. & Wilson, A. C. *Nucleic Acids Res.* **16**, 9775–9787 (1988).
4. Higuchi, R., Bowman, B., Friedberger, M., Ryder, O. A. & Wilson, A. C. *Nature* **312**, 282–284 (1984).
5. Smiley, C. J. (ed.) *Late Cenozoic History of the Pacific Northwest* (AAAS, San Francisco, California, 1985).
6. Niklas, K. J. in *Advances in Phytochemistry* (eds Reinhold, L., Harborne, J. B. & Swain, T.) **4**, 143–181 (Academic, London, 1980).
7. Niklas, K. J. in *Organic Maturation Studies and Fossil Fuel Exploration* (ed. Brooks, J.) 111–144 (Academic, London, 1981).
8. Niklas, K. J. in *Biochemical Aspects of Evolutionary Biology* (ed. Nitecki, M. H.) 29–91 (University of Chicago Press, Chicago, 1982).
9. Niklas, K. J., Brown, R. M., Jr & Santos, R. in *Late Cenozoic History of the Pacific Northwest* (ed. Smiley, C. J.) 143–159 (AAAS, San Francisco, 1985).
10. Rogers, S. O. & Bendich, A. J. *Plant molec. Biol.* **5**, 69–76 (1985).
11. Zurawski, G., Clegg, M. T. & Brown, A. H. D. *Genetics* **106**, 735–749 (1984).
12. Shinozaki, K. et al. *EMBO J.* **5**, 2043–2049 (1986).
13. Kreibers, E. T., Larrinua, I. M., McIntosh, L. & Bogorad, L. *Nucleic Acids Res.* **10**, 4985–5002 (1982).
14. Hiratsuka, J. et al. *Molec. gen. Genet.* **217**, 185–194 (1989).
15. Hattori, M. & Sakaki, Y. *Analyt. Biochem.* **152**, 232–238 (1986).
16. Kimura, M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 454–458 (1981).
17. Felsenstein, J. *PHYLIP 3.2 Manual* (University of California Herbarium, Berkeley, California, 1989).
18. Kishino, H. & Hasegawa, M. *J. molec. Evol.* **29**, 170–179 (1989).
19. Lampert, G. K., Elliot, L. V. & Bendich, A. J. *Planta* **148**, 437–443 (1980).
20. Smith, G. R. & Elder, R. L. in *Late Cenozoic History of the Pacific Northwest* (ed. Smiley, C. J.) 85–93 (AAAS, San Francisco, 1985).
21. Giannasi, D. E. & Niklas, K. J. *Science* **197**, 765–767 (1977).
22. Giannasi, D. E. & Niklas, K. J. *Am. J. Bot.* **68**, 762–770 (1981).
23. Giannasi, D. E. & Niklas, K. J. in *Late Cenozoic History of the Pacific Northwest* (ed. Smiley, C. J.) 161–174 (AAAS, San Francisco, 1985).
24. Rieseberg, L. H. & Soltis, D. E. *Biochem. Syst. Ecol.* **15**, 109–112 (1987).
25. Lin, C. M., Liu, Z. Q. & Kung, S. D. *Plant molec. Biol.* **6**, 81–87 (1986).
26. Aldrich, J., Cherney, B., Merlin, E. & Palmer, J. *Nucleic Acids Res.* **14**, 9534 (1986).
27. Zurawski, G., Perrot, B., Bottomley, W. & Whitfield, P. R. *Nucleic Acids Res.* **9**, 3251 (1981).
28. Aldrich, J., Cherney, B., Merlin, E. & Palmer, J. *Nucleic Acids Res.* **14**, 9535 (1986).
29. Zurawski, G., Whitfield, P. R. & Bottomley, W. *Nucleic Acids Res.* **14**, 3975–3976 (1986).
30. Kreitman, M. & Landweber, L. F. *Gene analyt. Techn.* **6**, 84–88 (1989).

ACKNOWLEDGEMENTS. We thank Mr Francis Kienbaum of Clarkia, Idaho, for permission and access to the P33 site, and Dr M. Sugiura for making a copy of the complete rice chloroplast genome sequence available to us. This work was supported in part by the NSF.

Cadmium and cobalt substitution for zinc in a marine diatom

N. M. Price & F. M. M. Morel

R. M. Parsons Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

IN the oceans, many trace metals show a surface depletion relative to deep waters that is typical of the principal algal nutrients, N, P and Si, and is therefore presumed to result from biological uptake at the sea surface and regeneration at depth. Among trace metals, cadmium has an especially acute surface depletion^{1,2}, and shows the best correlation with a major algal nutrient (P)^{1–3}. But the biological reason for Cd surface depletion is particularly puzzling, because unlike other surface-depleted trace elements (for example, Fe, Ni, Co, Zn and Cu), Cd is not known to be required by organisms. However, because Cd can substitute for Zn in some metalloenzymes *in vitro*⁴ and *in vivo*⁵, we hypothesized that Cd might promote the growth of Zn-limited phytoplankton. Marine phytoplankton are limited by a free Zn ion activity of 10^{–11.5} M (refs 6, 7), which is similar to the activity estimated for ocean surface waters⁸ as a result of the low concentration and organic complexation of Zn in the oceans. We now report that, in sea water with low Zn concentration, mimicking conditions of the ocean surface waters, Cd stimulates the growth of the marine diatom *Thalassiosira weissflogii* by substituting for Zn in certain macromolecules. The substitution is highly effective, in that Zn-deficient cells can grow at 90% of their maximum rate when supplied with Cd. We also find that Co can substitute for Zn (although less efficiently than Cd), indicating that Co could be an important nutrient for algal growth for reasons other than its role in vitamin B₁₂ (ref. 9).

We tested the effect of Cd and Co in bacteria-free cultures of the coastal diatom *T. weissflogii*, an organism for which Zn limitation has been documented. In the absence of Co, Cd and Zn, cell growth was impaired (Fig. 1). Zinc enrichment to

TABLE 1 Metal and protein in *T. weissflogii*

Element	Total metal	Soluble metal (amol per cell)	Insoluble metal	Protein (pg per cell)
Co	30.6	23.5	7.1	68.1
Cd	24.0 ± 3.0	8.6 ± 4.0	15.4 ± 1.1	43.7
Zn	66.4	16.6	49.8	41.2

Cellular concentrations of protein, and Co, Cd or Zn, in late exponential phase *T. weissflogii* grown in either Co-, Cd- or Zn-enriched medium. Cells were grown in medium (described in Fig. 1) containing carrier-free additions of either ^{57}Co (activity of $\text{Co}^{2+} = 10^{-10.9}\text{ M}$), ^{109}Cd (activity of $\text{Cd}^{2+} = 10^{-10.9}\text{ M}$) or ^{65}Zn (activity of $\text{Zn}^{2+} = 10^{-10.9}\text{ M}$). The cells were collected in late exponential phase by filtration onto 3.0- μm Nucleopore filters, and rinsed with 1 mM 8-hydroxyquinoline-5-sulphonate, a complexing agent that removes cell surface-adsorbed metal. Cells were then resuspended in buffer (0.01 M Tris buffer, 0.1 M KCl, 1 mM phenylmethylsulphonyl fluoride, pH 7) and disrupted by sonication. Protein content was determined by the method of Lowry¹⁵, and radioactivity was measured by liquid scintillation counting, using an external standard to correct for quenching. Broken-cell extracts were centrifuged at 4 °C in a Beckman L8-55 ultracentrifuge with a 70.1 Ti Rotor at 120,000g (40,000 r.p.m.) for 2 h. The amount of metal in the supernatant (soluble metal) was measured, and the amount in the pellet (insoluble metal) was calculated by difference. The quantity of Cd per cell is an average result of duplicate experiments ± 1 s.d. Cells contained about 10 pmol C per cell (determined using H^{14}CO_3), equivalent to about 0.1 pmol P per cell.

$10^{-10.9}\text{ M}$ free Zn (the normal culture value) increased cell growth rate to a maximum of 3.3 divisions per day. Identical results were obtained for cultures supplied with any combination of these three metals, provided that Zn was added. Addition of Co alone, at the same free metal activity as Zn, increased cell growth rate, but only to 60% of that observed in the presence of Zn. With similar addition of Cd (activity of $\text{Cd}^{2+} = 10^{-10.9}\text{ M}$), cells grew at rates that were 90% of control (Zn added) cultures (Fig. 1). Cells supplied with Co and Cd grew no better than those supplied with only Cd.

The amount of Zn contained in cells of Zn-enriched cultures was twice the amount of either Co or Cd contained in cells of Co- or Cd-enriched cultures, respectively (Table 1). The additional Zn was accounted for in the insoluble fraction, representing chiefly the membranous constituents. For each of the enriched cultures, gel filtration of the soluble cell extract (Fig. 2a) revealed two peaks of metal concentration, neither of which coincided with the 280-nm-absorbing material (Fig. 2b). The larger species (relative molecular mass, M_r , 66,000 (66K)) eluted as a similarly broad band for all three metals. The species with lower M_r , which was predominant with Co, eluted near the permeation volume and was roughly of M_r 1.5K.

We attempted to characterize these soluble metal species. The 66K fraction is likely to represent some Zn metalloenzymes, but we have not yet characterized them. Our results seem to rule out carbonic anhydrase¹⁶ because of its larger M_r , and alkaline phosphatase^{17,18}, because its activity was independent of the additions of trace metals. This last observation is relevant in view of the remarkable Cd- PO_4 correlation in the ocean. The M_r of the low- M_r metal fraction (1.5K) is near that of phytochelatin in higher plants and algae^{19,20}. We confirmed that, as is characteristic of phytochelatin, this fraction is heat-stable (70 °C for 5 min), contains a high concentration of sulphur (50% of the soluble cellular S), and binds metals *in vitro* as well as *in vivo*. It is notable that this phytochelatin-like fraction is present here under conditions of very low metal concentrations, rather than metal toxicity. We suggest that, in marine phytoplankton, phytochelatin serves as intracellular metal buffers under all conditions, including metal deficiency as well as excess. This is consistent with the recent report that phytochelatin synthetase is constitutive and regulated by metals²¹.

When both Cd and Zn were present in the medium, Cd cell quota was 10 amol (10^{-18} mol) per cell and the soluble Cd was chiefly in the low- M_r material (Fig. 3). Cadmium quota increased to 24 amol Cd per cell when Zn was omitted from the medium, indicating a greater cellular demand for Cd under Zn-limitation. Under these conditions, the Cd concentration doubled in the low- M_r material and at least tripled in the high- M_r fraction.

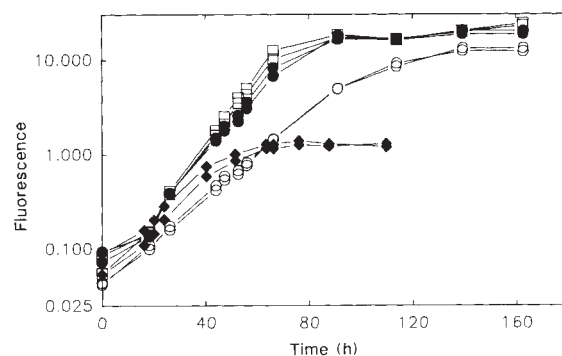


FIG. 1 Growth of *T. weissflogii* in Aquil medium¹⁰ containing either Co (○), Cd (●), Zn (□), or none of these metals (◆). All media were initially enriched with an EDTA/trace-metal solution containing 100 μM EDTA, with Fe, Cu and Mn concentrations adjusted to achieve free-ion activities of $10^{-18.2}\text{ M}$, $10^{-13.9}\text{ M}$ and $10^{-8.37}\text{ M}$. These activities were determined by calculation, using the chemical equilibrium program MINEQL¹¹, with thermodynamic constants obtained from Ringbom¹². Cobalt, Cd and Zn were added as EDTA complexes (1:1) to achieve free-ion activities of $10^{-10.9}\text{ M}$. Cells were cultured in silanized borosilicate tubes under a constant photon flux density of $150\text{ }\mu\text{E m}^{-2}\text{ s}^{-1}$ at 20 °C. Growth was measured by *in vivo* chlorophyll fluorescence¹³. Vitamin (B_{12} , thiamine and biotin) and EDTA/trace-metal additions were filter-sterilized (0.2 μm) and then added to sterile media¹⁴.

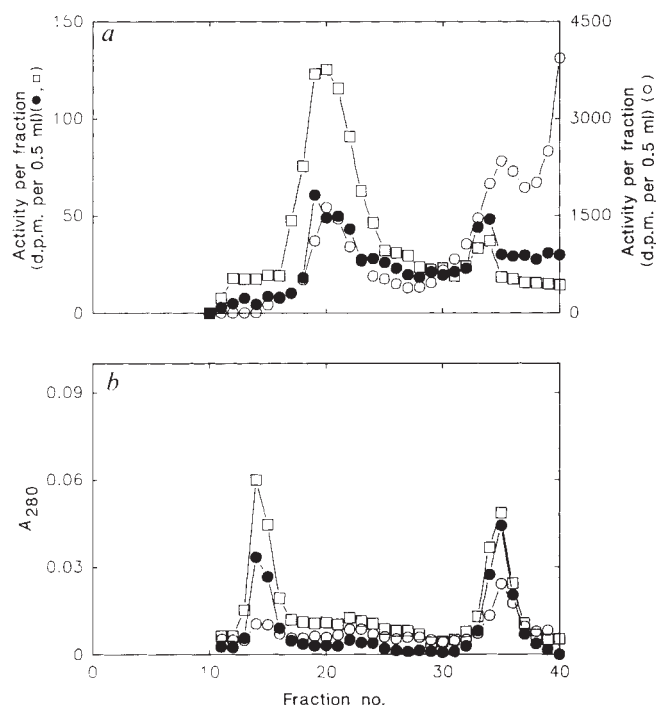


FIG. 2 Chromatography of soluble macromolecules of *T. weissflogii* labelled with ^{57}Co (○), ^{109}Cd (●) and ^{65}Zn (□) as described in Table 1 on a Sephacryl S-200 column (1.5 $\times$ 50 cm). The samples were eluted with 0.01 M Tris-0.1 M KCl buffer (pH 7), and fractions assayed for radioactivity (a) and absorbance at 280 nm (A_{280}) (b). Standards of known M_r were used to calibrate the column: cytochrome c, carbonic anhydrase, albumin, alcohol dehydrogenase, and β -amylase. The samples were also chromatographed on a Sephadex G-25 column (1.5 $\times$ 50 cm) to estimate the M_r of the small metal-containing material (data not shown).

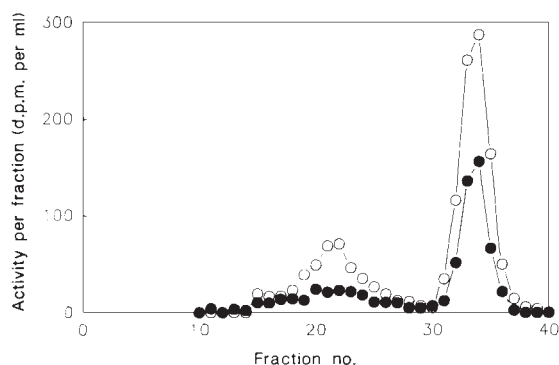


FIG. 3 Elution profiles of soluble ^{109}Cd -labelled compounds from cells grown in media containing either ^{109}Cd (activity of $\text{Cd}^{2+} = 10^{-10.9}\text{ M}$) and Zn (activity of $\text{Zn}^{2+} = 10^{-10.9}\text{ M}$) (●) or ^{109}Cd (activity of $\text{Cd}^{2+} = 10^{-10.9}\text{ M}$) only (○). Soluble extracts were prepared as described in Table 1. Equal quantities of protein (60 μg) from each sample were applied to a Sephacryl S-200 column and the radioactivity in the eluted fractions assayed.

The Zn cell quota was the same regardless of whether or not Cd was supplied to the medium.

Three lines of evidence demonstrate that Co and Cd substitute for Zn in *T. weissflogii*. First, the growth-promoting properties of Co and Cd are only evident when Zn is absent from the medium. Second, the distribution of Co, Cd and Zn among the soluble cellular constituents is remarkably similar (leading us to believe that we are observing not an enzyme substitution but a metal substitution in the same protein). Finally, the quantity of Cd per cell decreases in the presence of Zn and increases in the absence of Zn.

Normalized to phosphorus, the cellular concentration of Cd we obtained in our experiments is in the range of those in surface seawater particles (Cd/P , $0.1\text{--}1.0 \times 10^{-3}$; refs 3, 22). Therefore, the biological use of Cd by phytoplankton can apparently account for its oceanographic distribution. The simultaneous depletion of several trace elements in surface sea water can be explained in part by biochemical metal substitution as exemplified here by Co, Cd and Zn. Substitution of trace metals or metalloenzymes could be a common strategy for phytoplankton survival in trace metal-improverished environments, such as the ocean, and could result in an effective colimitation of phytoplankton growth by several bioactive elements. □

Received 11 December 1989; accepted 6 February 1990.

- Boyle, E., Sciater, F. R. & Edmond, J. M. *Nature* **263**, 42–44 (1976).
- Bruland, K. W., Knauer, G. A. & Martin, J. H. *Limnol. Oceanogr.* **23**, 618–625 (1978).
- Martin, J., Bruland, K. W. & Broenkow, W. in *Marine Pollutant Transfer* (eds Windom, H. L. & Duce, R. A.) 159–184 (Heath, Lexington, Massachusetts, 1976).
- Bertini, I. & Luchinat, C. in *Metal Ions in Biological Systems* Vol. 15 (ed. Sigel, H.) 101–156 (Dekker, Basle, 1983).
- Rosenbusch, J. P. & Weber, K. *Proc. natn. Acad. Sci. U.S.A.* **68**, 1019–1023 (1971).
- Anderson, M. A., Morel, F. M. M. & Guillard, R. R. L. *Nature* **276**, 70–71 (1978).
- Brand, L. E., Sunda, W. G. & Guillard, R. R. L. *Limnol. Oceanogr.* **28**, 1182–1195 (1983).
- Bruland, K. W. *Limnol. Oceanogr.* **34**, 269–285 (1989).
- Babor, B. M. (ed.) *Cobalamin: Biochemistry and Pathophysiology* (Wiley, New York, 1975).
- Morel, F. M. M., Reuter, J. G., Anderson, D. M. & Guillard, R. R. L. *J. Phycol.* **15**, 135–141 (1979).
- Westall, J. C., Zachary, J. L. & Morel, F. M. M. *MINEQL: A Computer Program for the Calculation of Chemical Equilibrium Composition of Aqueous Systems* (Department of Civil Engineering, MIT, Cambridge, Massachusetts, 1976).
- Ringbom, A. *Complexation in Analytical Chemistry* (Interscience, New York, 1963).
- Brand, L. E., Guillard, R. R. L. & Murphy, L. S. *J. Plankton Res.* **3**, 193–201 (1981).
- Keller, M. D., Bellows, W. K. & Guillard, R. R. L. *J. exp. mar. Biol. Ecol.* **117**, 279–283 (1988).
- Lowry, O. H. et al. *J. biol. Chem.* **193**, 265–275 (1951).
- Graham, D., Reed, M. L., Patterson, B. D., Hockley, D. G. & Dwyer, M. R. in *Biology and Chemistry of the Carbonic Anhydrases* (eds Tashian, R. E. & Hewett-Emmett, D.) 222–237 (New York Academy of Sciences, New York, 1984).
- Vallee, B. L. & Galde, A. *Adv. Enzym.* **56**, 283–430 (1984).
- Perry, M. J. *Mar. Biol.* **15**, 113–119 (1972).
- Gekeler, W., Grill, E., Winnacker, E.-L. & Zenk, M. H. *Arch. Microbiol.* **150**, 197–202 (1988).
- Grill, E., Winnacker, E.-L. & Zenk, M. H. *Proc. natn. Acad. Sci. U.S.A.* **84**, 439–443 (1987).
- Grill, E., Löffler, S., Winnacker, E.-L. & Zenk, M. H. *Proc. natn. Acad. Sci. U.S.A.* **86**, 6838–6842 (1989).
- Sherrell, R. M. thesis, MIT (1989).

ACKNOWLEDGEMENTS. We thank S. W. Chisholm, B. Palenik and J. A. Raven for comments on the manuscript. This work was supported by the NSF and the ONR. N.M.P. was funded by the NSERC of Canada and the Killam foundation.

Rarity of density dependence or population regulation with lags?

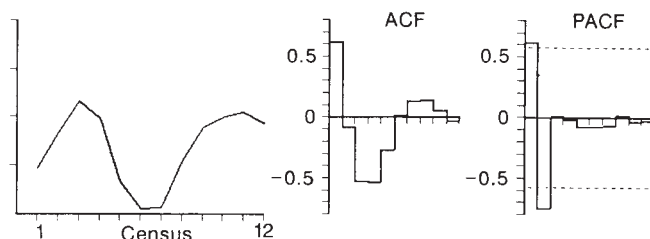
Peter Turchin

Southern Forest Experiment Station, 2500 Shreveport Hwy, Pineville, Louisiana 71360, USA

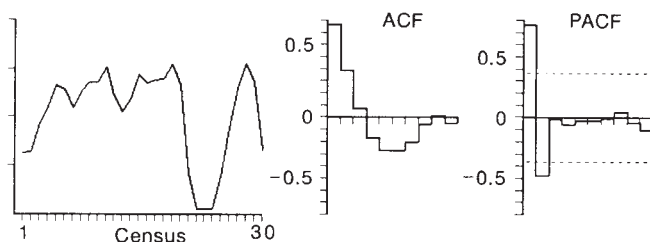
SEVERAL recent reviews of published life tables^{1–3} concluded that density-dependent regulation is infrequent in insect populations, prompting a vigorous debate among ecologists^{4–10}. Little attention, however, has been directed to one issue: most life-table analyses look only for direct (not-lagged) density dependence. Thus, there is a real danger that populations characterized by delays in regulation will be relegated to a density-independent limbo by an analysis not equipped to recognize such behaviour. I have evaluated the evidence for delayed density dependence in population dynamics of 14 forest insects, and assessed the effect of regulation lags on the likelihood of detecting direct density dependence. Eight cases exhibited clear evidence for delayed density dependence and lag-induced oscillations, but direct density dependence was detected in only one of these. This result suggests that traditional analyses will not, in general, detect density-dependent regulation in populations that are characterized by lags and complex dynamic behaviour.

In selecting case studies for the analysis, my focus was exclusively on temporal density dependence, which is the central issue in the population regulation controversy^{5,6}. Accordingly, I looked for temporal records of forest insect populations from a single locality. When there had been a census on the same species in several locations, or there had been several studies on it, I selected the longest time-series available. I used all the records I could find of more than 10 consecutive censuses.

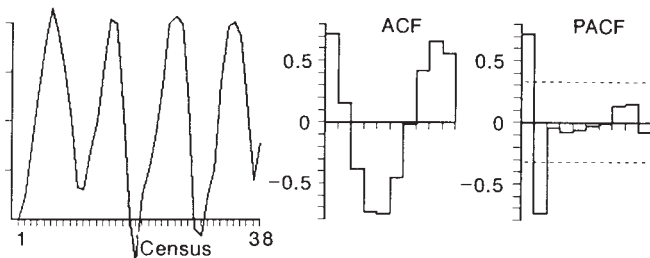
a *Acleris variana*



b *Dendroctonus frontalis*



c *Zeiraphera diniana*



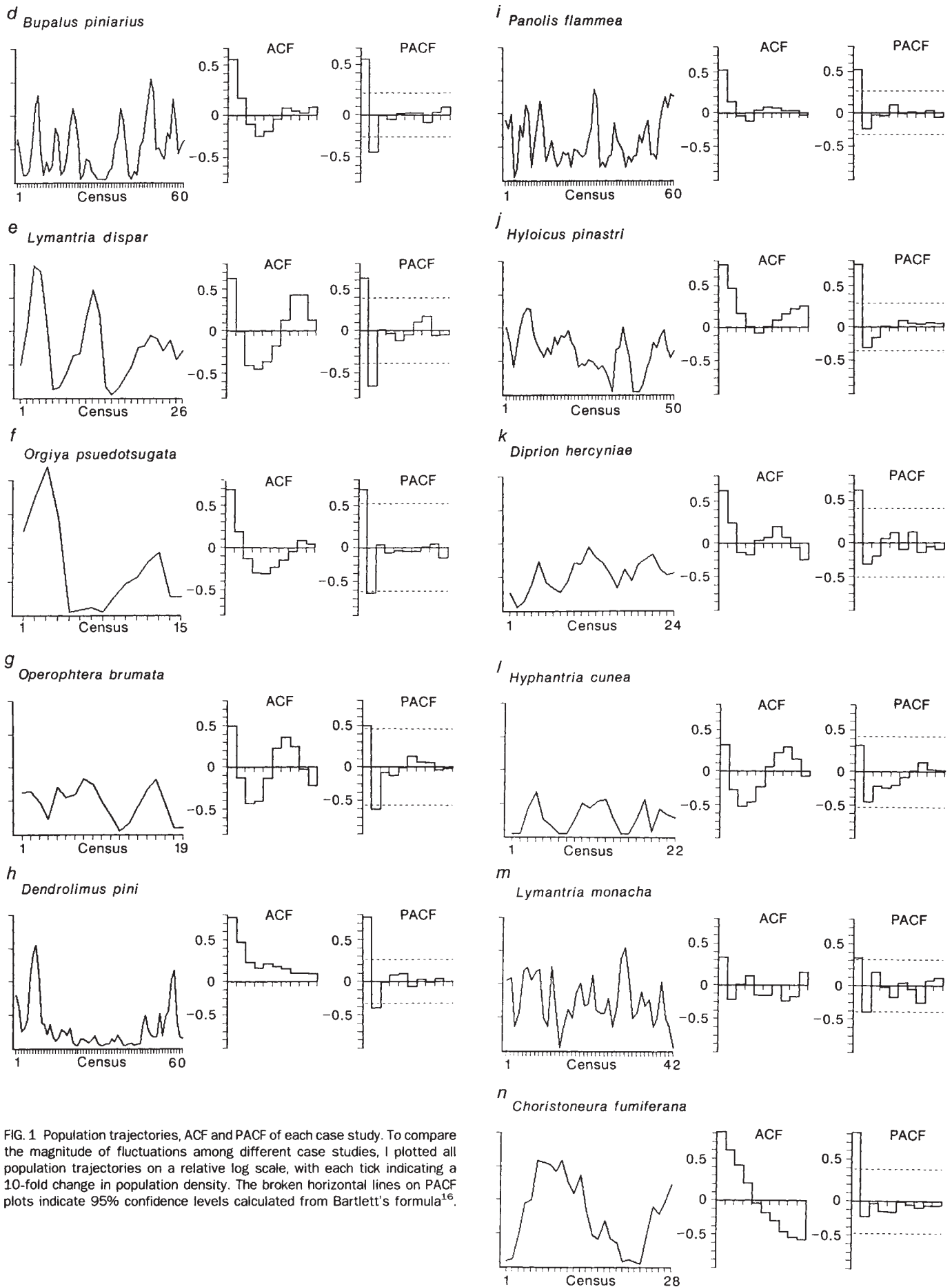


FIG. 1 Population trajectories, ACF and PACF of each case study. To compare the magnitude of fluctuations among different case studies, I plotted all population trajectories on a relative log scale, with each tick indicating a 10-fold change in population density. The broken horizontal lines on PACF plots indicate 95% confidence levels calculated from Bartlett's formula¹⁶.

TABLE 1 Results of fitting population data with model 2

Species	r_0	α_1	α_2	Sign	R^2
a. <i>Acleris variana</i> ²⁰	0.66 (0.63) 1.26 (0.35)	-0.0038 (0.003) -0.0015 (0.0015)		NS ***	0.193 0.811
b. <i>Dendroctonus frontalis</i> [†]	0.66 (0.44) 1.30 (0.35)	-0.00028 (0.00014) -0.00004 (0.00012)	-0.0073 (0.0015)	NS ***	0.135 0.552
c. <i>Zeiraphera diniana</i> ²¹	0.54 (0.49) 1.20 (0.39)	-0.008 (0.004) -0.0001 (0.004)	-0.020 (0.004)	NS **	0.087 0.511
d. <i>Bupalus piniarius</i> ²²	0.20 (0.22) 0.34 (0.21)	-0.0013 (0.0005) -0.0005 (0.0006)	-0.0018 (0.0006)	* **	0.091 0.241
e. <i>Lymantria dispar</i> ²³	0.12 (0.44) 0.40 (0.36)	-0.00008 (0.00006) -0.000002 (0.00006)	-0.00020 (0.00005)	NS ***	0.074 0.426
f. <i>Orgyia pseudotsugata</i> ²⁴	-0.25 (0.67) 0.31 (0.40)	-0.008 (0.006) -0.007 (0.004)	-0.018 (0.004)	NS ***	0.125 0.739
g. <i>Operophtera brumata</i> ²⁵	0.37 (0.37) 0.92 (0.37)	-0.0054 (0.0030) -0.0035 (0.0026)	-0.0074 (0.0027)	NS *	0.183 0.472
h. <i>Dendrolimus pini</i> ²⁶	0.03 (0.15) 0.10 (0.13)	-0.0010 (0.0005) +0.0002 (0.0006)	-0.0023 (0.0006)	NS ***	0.062 0.268
i. <i>Panolis flammea</i> ²²	0.21 (0.13) 0.30 (0.12)	-0.041 (0.014) -0.026 (0.014)	-0.040 (0.015)	** *	0.141 0.232
j. <i>Hyloicus pinastri</i> ²²	0.09 (0.09) 0.14 (0.09)	-0.025 (0.011) +0.007 (0.016)	-0.044 (0.016)	* **	0.092 0.222
k. <i>Diprion hercyniae</i> ²¹	0.57 (0.24) 0.67 (0.27)	-0.0021 (0.0008) -0.0017 (0.0009)	-0.0008 (0.0009)	* NS	0.252 0.286
l. <i>Hyphantria cunea</i> ²⁷	0.69 (0.22) 0.85 (0.25)	-0.13 (0.03) -0.13 (0.03)	-0.04 (0.03)	*** NS	0.473 0.514
m. <i>Lymantria monacha</i> ²⁸	0.06 (0.45) 0.32 (0.46)	-0.00003 (0.00002) -0.00002 (0.00002)	-0.00004 (0.00002)	NS NS	0.055 0.125
n. <i>Choristoneura fumiferana</i> ¹¹	0.29 (0.19) 0.29 (0.20)	-0.018 (0.009) -0.016 (0.011)	-0.002 (0.012)	NS NS	0.139 0.140

The first line in each case study shows the results of regression on N_{t-1} only, and indicates whether this regression was significant (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant). The second line shows the results of adding N_{t-2} to the regression in a step-wise fashion and testing whether the increase in regression sum of squares is significant. The numbers in parentheses are the standard errors of the estimates.

[†] P.T. and R. Billings, manuscript in preparation.

Delayed density dependence can arise in natural populations as a result of interactions with other members of the community, such as natural enemies^{8,11}, or because high population density may adversely affect the fecundity of the next generation¹². Several methods for detecting delayed regulation have been suggested¹³⁻¹⁵. I used two distinct approaches, each providing a different viewpoint of the temporal population dynamics. First, I used the diagnostic techniques of time-series analysis¹⁶. In particular, for each record of population fluctuations I estimated the autocorrelation function (ACF) and the partial autocorrelation function (PACF). The strength of the Box-Jenkins approach lies in its ability to consider multiple lag effects. Its weakness, with respect to population modelling, is its assumption that log-transformed population densities are related in a linear fashion, for example, in a form of an autoregressive process of the order p , AR(p)

$$L_t = a_0 + a_1 L_{t-1} + \dots + a_p L_{t-p} + \varepsilon_t \quad (1)$$

Here, $L_t = \log N_t$ is the log-transformed population density at census t ; $a_1, \dots, a_p$ are weights that quantify the influence of past densities on the population change, and ε_t is a random variable with mean zero and variance σ^2 . As real-world population dynamics are inherently non-linear¹¹ the Box-Jenkins approach can be useful for identification of the broad patterns in the data, but not for modelling the population fluctuations.

The second approach involved specifying a non-linear model for N_t and considering a limited number of lags. I used the following phenomenological model, which is an extension of the Ricker equation,

$$N_t = N_{t-1} \exp [r_0 + \alpha_1 N_{t-1} + \alpha_2 N_{t-2} + \varepsilon_t] \quad (2)$$

The parameters of the model r_0 , α_1 , α_2 , and σ^2 (the variance of the exogenous component ε_t) are readily estimated by regressing the rate of population change $r \equiv \log(N_t/N_{t-1})$ on N_{t-1} and N_{t-2} . This procedure assumes that errors are multiplicative and distributed log-normally, which is the standard assumption

in statistical analyses of population fluctuations^{11,17}. I analysed each series of population censuses using a stepwise regression: first regressing r on N_{t-1} , and then testing whether adding the term N_{t-2} significantly reduced unexplained variance.

Strong evidence for delayed density-dependent regulation was found in eight out of 14 population trajectories ($a-h$ in Fig. 1 and Table 1). Consider the ACF/PACF graphs first. The PACF is known to be a particularly useful diagnostic tool for determining the order of an AR process¹⁶. The theoretical PACF of the first-order autoregressive process, AR(1), is characterized by a single spike at lag 1, and is zero everywhere else¹⁶ (compare with the PACF shapes for cases i and k). In cases $a-h$, however, PACFs have two spikes (at lags 1 and 2) that are significantly different from zero (Fig. 1). Such a pattern is consistent with the theoretical PACF of AR(2) (ref. 16). Note that PACF at lag 2 is negative. A negative term involving N_{t-2} in equation (1) implies delayed density regulation. The ACF patterns in these cases also exhibited a great degree of similarity (Fig. 1). In all cases but h , the ACF behaved as a damped sine wave, which indicates an oscillatory endogenous component in the dynamics of populations $a-g$ ¹⁸.

Fitting the data from these eight case studies with equation (2) showed that including N_{t-2} significantly improved the fit in all eight cases (Table 1). This result corroborates the conclusions drawn from the time-series analysis. Regression on N_{t-1} alone, however, was significant only in case d .

One potential problem in interpreting results of regression on r is that sampling errors in N_t bias the estimates of the slopes α_1 and α_2 . Walters and Ludwig¹⁹ showed that, for the Ricker model, the bias in the slope estimate depends on the magnitude of the true slope. If density regulation is weak or absent, then the effect of observation errors is to overestimate the amount of density dependence. On the other hand, if regulation is strong, then density dependence will be underestimated. This bias, however, does not affect my main conclusion, as it is based on a contrast between the estimated effects of direct versus delayed density dependence. In fact, observation errors make such con-

trasts conservative, as in $a-h$ direct density dependence is weak and will therefore be overestimated, whereas delayed density dependence is strong and therefore underestimated.

For the other cases, $i-n$, PACF showed no evidence of delayed density-dependent regulation. But in two cases, i and j , including N_{t-2} significantly improved the fit to model (2). In cases k and l only evidence for direct density-dependence was found, whereas cases m and n showed no evidence for density-dependent regulation, either direct or delayed (Table 1).

In conclusion, an analysis that does not consider time lags will not, in general, detect density-dependent regulation in population oscillations driven by delayed density-dependent mechanisms. The analysis in this paper detected direct density dependence in only 5 out of 14 populations. This proportion, 36%, is not that different from similar proportions reported in previous surveys¹⁻³. But further analysis showed that seven out of the nine apparently non-regulated populations were, in fact, subject to delayed density-dependent regulation. Thus, it is conceivable that many insect populations classified as density-independent by Stiling³ are actually regulated by delayed density-dependent mechanisms. □

Received 18 December 1989; accepted 1 February 1990.

1. Dempster, J. P. *Biol. Rev. Cambridge. Phil. Soc.* **58**, 461-481 (1983).

2. Stiling, P. *Ecology* **68**, 844-856 (1987).
3. Stiling, P. *J. Anim. Ecol.* **57**, 581-594 (1988).
4. Hassell, M. P. *J. Anim. Ecol.* **54**, 323-334 (1985).
5. Dempster, J. P. & Pollard, E. *Oikos* **46**, 413-416 (1986).
6. Hassell, M. P. *J. Anim. Ecol.* **56**, 705-713 (1987).
7. Strong, D. *Trends Ecol. Evol.* **1**, 39-42 (1986).
8. Murdoch, W. W. & Reeve, J. D. *Oikos* **50**, 137-141 (1987).
9. Brown, M. W. *Ecology* **70**, 776-779 (1989).
10. Stiling, P. *Ecology* **70**, 783-786 (1989).
11. Royama, T. *Ecol. Monogr.* **51**, 473-493 (1981).
12. Prout, T. & McChesney, F. *Am. Nat.* **126**, 521-558 (1985).
13. Moran, P. A. P. *Aust. J. Zool.* **1**, 163-173 (1953).
14. Royama, T. *Ecol. Monogr.* **47**, 1-35 (1977).
15. Berryman, A. A. *Can. Ent.* **110**, 513-518 (1978).
16. Box, G. E. P. & Jenkins, G. M. *Time Series Analysis, Forecasting and Control* (Holden Day, Oakland, 1976).
17. Pollard, E., Lakhani, K. H. & Rothery, P. *Ecology* **68**, 2046-2055 (1987).
18. Nisbet, R. M. & Gurney, W. S. C. *Modelling Fluctuating Populations* (Wiley, Chichester, 1982).
19. Walters, C. J. & Ludwig, D. *Can. J. Fish. Aquat. Sci.* **38**, 704-710 (1987).
20. Morris, R. F. *Ecology* **40**, 580-588 (1959).
21. Baltensweiler, W. & Fischlin, A. in *Dynamics of Forest Insect Populations* (ed. Berryman, A. A.) 332-353 (Plenum, New York, 1988).
22. Schwerdtfeger, F. *Z. Angew. Ent.* **28**, 254-303 (1941).
23. Montgomery, M. E. & Wallner, W. E. in *Dynamics of Forest Insect Populations* (ed. Berryman, A. A.) 354-377 (Plenum, New York, 1988).
24. Mason, R. R. & Wickman, B. E. in *Dynamics of Forest Insect Populations* (ed. Berryman, A. A.) 180-211 (Plenum, New York, 1988).
25. Varley, G. C., Gradwell, G. R. & Hassell, M. P. *Insect Population Ecology: An Analytical Approach* (University of California Press, Berkeley, 1974).
26. Varley, G. C. *J. Anim. Ecol.* **18**, 117-122 (1949).
27. Morris, R. F. *Can. Ent.* **96**, 356-368 (1964).
28. Bejer B. in *Dynamics of Forest Insect Populations* (ed. Berryman, A. A.) 212-233 (Plenum, New York, 1988).

ACKNOWLEDGEMENTS. I thank A. Berryman, W. Morris, T. Royama and A. Taylor for discussion and editorial comment.

Population structure of the human pseudoautosomal boundary

Nathan Ellis*, Anne Taylor*, Bengt O. Bengtsson†, Judy Kidd‡, Jeffrey Rogers‡ & Peter Goodfellow*

* Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, UK

† Department of Genetics, Lund University, Lund, Sweden

‡ Department of Human Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510, USA

THE mammalian sex chromosomes are composed of two genetically distinct segments: the pseudoautosomal region, where recombination occurs between the X and Y chromosomes, and the sex chromosome-specific parts^{1,2}. Between these two segments the human sex chromosomes differ by the insertion of an *Alu* element on the Y chromosome³. We have surveyed the sequence variation in the boundary region using the polymerase chain reaction. Fifty seven Y and sixty X chromosomes from ten different human populations were analysed. The X chromosomes were found to be polymorphic at five positions in a 300-base-pair region. By contrast, all Y chromosomes were identical except for one distal polymorphism shared with the X chromosome.

We used direct sequencing and restriction mapping of DNA amplified by the polymerase chain reaction (PCR) to survey the variation between the sex chromosomes in the boundary region (Fig. 1). Position numbers were assigned by defining the *Alu* insertion site as 0 and naming the first X-Y homologous base XY1, the first Y-specific base Y1, and the first X-specific base X1. Variation at positions XY41, XY45 and XY274 has previously been described^{3,4}. New variants detected in this study were found at positions X35, XY71, XY161 and XY171 (Table 1).

All the Y chromosomes were identical except for the most distal locus, XY274, where polymorphism was found in some African populations. The X chromosomes, however, showed rich genetic variation, with polymorphism detected at X35, XY71, XY161, XY171 and XY274.

At the three central loci, XY71, XY161 and XY171, two haplotypes predominated, TAT and GGA, with a third haplotype, TGA, found only in South African Bantu-speaking blacks and San (Kalahari/Kung formerly 'Bushmen'). Using data from northern Europeans, Table 2 compares the alleles at X35 and XY274 with the alleles at the three central loci as represented by XY71. Significant linkage disequilibrium was found between X35 and XY71 and between XY71 and XY274, but not between the most distant loci X35 and XY274. Table 3 summarizes the haplotype analysis of northern Europeans; of the 32 possible haplotypes, only five were found in the sample of 19 X chromosomes. More variation was found in the African San population, where five haplotypes were detected in only nine X chromosomes tested.

All Y chromosomes are different from the X chromosomes at sites XY41 and XY45. From position XY46 to XY171 the Y chromosomes have the same sequence as a common X chromosome haplotype. At position XY274 the two sex chromosomes share the same polymorphism.

The insertion of the *Alu* element must have been a unique event and all Y chromosomes in higher apes descended from the chromosome in which it happened³. The divergence of the X and Y chromosomes proximal to the insertion site implies that recombination has not occurred in this region since the time of the *Alu* insertion³. The same reduced similarity would be expected in the region distal to the *Alu* insertion if there had not been any genetic contact between the X and Y chromosomes. But it has previously been shown that the sex chromosomes are highly similar in this region³, and in the present study we find strong evidence for recombination occurring proximal to XY274. The shared polymorphism on the X and Y chromosomes at this site would otherwise have to be explained by coincident mutations. The crossover could have occurred at any position distal to XY45, as in this region all Y chromosomes are identical with a common X chromosome haplotype. Only at positions XY41 and XY45 do we find divergence between the X and Y chromosomes. We conclude that the boundary between the sex specific and the pseudoautosomal parts of the X and Y chromosomes is at the *Alu* insertion site or very close to it.

The sequence variation found in the boundary region must be the outcome of an interplay between many evolutionary factors, including mutation, selection, recombination, population

TABLE 1 Haplotypes from Y and X pseudoautosomal boundaries

	Y chromosomes						<i>n</i>
	XY41	XY45	XY71	XY161	XY171	XY274	
Northern European	C	A	T	A	T	T	21
	C	A	T	A	T	ND	1
Japanese	C	A	T	A	T	T	5
Ro. Surui*	C	A	T	A	T	T	5
Karitiana*	C	A	T	A	T	T	4
Quechua*	C	A	T	A	T	T	2
Ethiopian	C	A	T	A	T	T	2
	C	A	T	A	T	C	1
	C	A	T	A	T	ND	1
Pygmy	C	A	T	A	T	T	3
Southern African Bantu†	C	A	T	A	T	T	4
San‡	C	A	T	A	T	T	4
	C	A	T	A	T	C	2
Papua§	C	A	T	A	ND	T	1
	C	A	T	A	T	ND	1
	X chromosomes						<i>n</i>
	X35	XY41	XY45	XY71	XY161	XY171	
Northern European	T	G	T	T	A	T	5
	G	G	T	T	A	T	4
	G	G	T	T	A	T	3
	T	G	T	G	G	A	3
	G	G	T	T	A	ND	2
	G	G	T	T	A	T	1
	T	G	T	G	G	ND	1
	T	G	T	G	ND	ND	1
	ND	G	T	T	A	T	1
	T	G	T	G	G	A	1
Japanese	T	G	T	G	G	A	2
	G	G	T	T	A	T	1
	G	G	T	T	A	T	1
Ro. Surui*	G	G	T	T	A	T	2
	G	G	T	T	A	ND	1
	ND	G	T	T	A	ND	1
Karitiana*	G	G	T	T	A	T	5
Quechua*	G	G	T	T	A	T	3
Ethiopian	G	G	T	T	A	T	1
	G	G	T	T	A	T	1
	T	G	T	T	A	ND	1
	T	G	T	T	A	T	1
Pygmy	G	G	T	T	A	T	1
	T	G	T	G	G	A	1
	T	G	T	T	A	T	1
Southern African Bantu†	G	G	T	T	G	A	2
	T	G	T	T	A	T	1
San‡	T	G	T	G	G	A	3
	G	G	T	T	A	T	3
	G	G	T	T	A	T	1
	T	G	T	G	G	A	1
	G	G	T	T	G	A	1
Papua§	G	G	T	T	A	T	2
	G	G	T	T	A	T	1

ND, samples not determined at that locus; *n*, no. of individuals.

* South American Amerindian tribe.

† Southern African Bantu-speaking black.

‡ Kalahari/Kung San, formerly 'Bushmen'.

§ These Papuans are from the western Highlands and are Australoid in origin.

|| These individuals carry a deletion of ACTA (or AACT or CTAA) at XY22.

TABLE 2 Two-by-two matrices for three polymorphic sites in northern European X chromosomes

1.		2.		3.	
XY71		XY274		XY274	
	T G		C T		C T
X35 G	10 0	XY71 T	5 10	X35 G	5 4
	T 5 6		G 5 1		T 5 6

The middle three loci are presented by the locus XY71.

TABLE 3 Haplotype analysis of 21 Y and 19 X chromosomes in northern Europeans

	X35	XY71	XY161	XY171	XY274	<i>n</i>
Y chromosomes	NA	T	A	T	T	21
X chromosomes	T	G	G	A	C	4
	T	G	G	A	T	1
	T	T	A	T	T	5
	G	T	A	T	C	5
	G	T	A	T	T	4

Individuals who were not determined at one of the middle three loci were assumed to have TAT or GGA on the basis of the other two loci.

NA, not applicable.

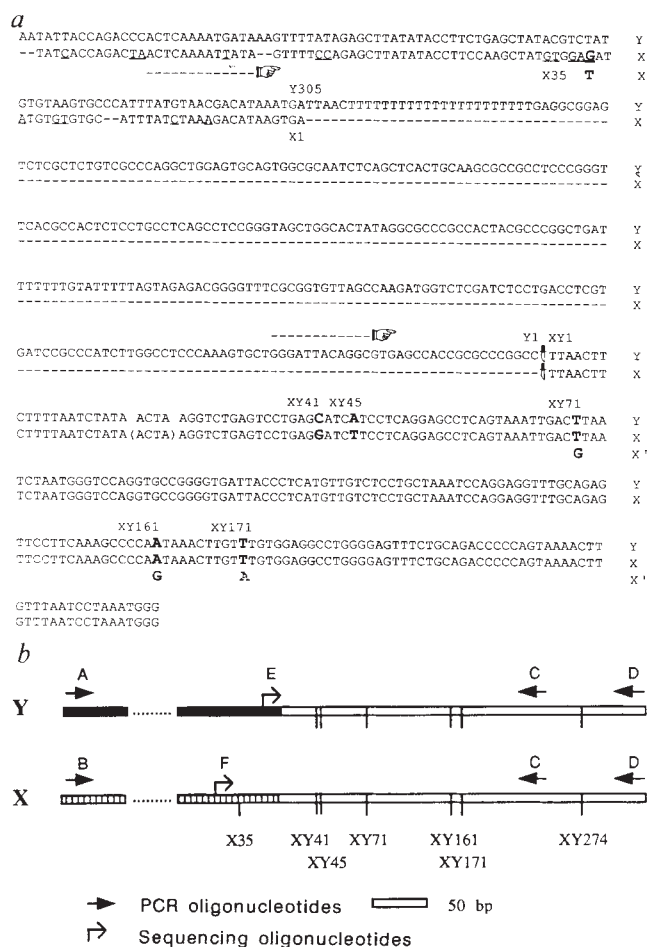


FIG. 1 *a*, Sequences found in the pseudoautosomal boundary region on the X and Y chromosomes³. A 303-bp *Alu* repeat element is inserted on the Y chromosome. On the telomeric side of the *Alu* element, sequences are strictly homologous (>99%) between the sex chromosomes. Proximal to the *Alu* element, a region of partial homology (77%) extends for 225 bp to a point where homology is lost completely. The polymerase chain reaction (PCR) was performed on male DNA with a 30-mer derived from pseudoautosomal sequences (oligo C in *b*) in conjunction with a 30-mer from Y-specific (oligo A in *b*) or X-specific (oligo B in *b*) regions. Products were identified as a 947-bp fragment from the Y chromosome, and a 771-bp fragment from the X chromosome. Sequences were obtained by reading from the insertion site into pseudoautosomal sequences with either of two primers: one Y-specific (oligo E in *b*) and one X-specific (oligo F in *b*). Hand symbols show where sequencing begins. All sequences read across the *Alu* insertion site (represented by daggers) into pseudoautosomal sequences. Positions are numbered with the *Alu* insertion site as position zero. The length of the reading ranged from 180 bp to >250 bp. Sequencing identified variation between chromosomes at X35, XY41, XY45, XY71, XY161 and XY171. Variant positions are in bold with the alternative allele below. A 4-bp deletion at XY22 was found in some San X chromosomes (parentheses).

METHODS. PCR was according to standard Cetus conditions^{8,9} with 30 cycles at 94 °C for 1 min, 54 °C for 1 min, and 72 °C for 2 min. A final incubation at 72 °C for 6 min was added to the last cycle. Amplified product was purified by phenol extraction and spermine precipitation¹⁰ then resuspended in a small volume. Sequencing primer was end-labelled by phosphorylation of the 5'-OH with [γ -³²P]ATP in a reaction using T4 polynucleotide kinase¹¹. Primer (5–10 pmol) was annealed to one half or all of the amplified product for sequencing by the dideoxy-chain-termination method¹². *b*, Schematic representation of all loci at which base pair variation between chromosomes was found. XY274 was assayed by PCR with a 21-mer derived from pseudoautosomal sequences (oligo D) in conjunction with either X- or Y-specific 30-mers. PCR product was digested with *MspI* and separated by 1% agarose gel electrophoresis. The XY274 C-bearing allele is identified by 156-bp and 180-bp *MspI* fragments, whereas the XY274 T-bearing allele is identified by a 336-bp *MspI* fragment⁴. Oligonucleotide sequences: A, GTACTACCTTTAGAAAAGTATTTTCCC; B, CTGCAGAAACAAGCTCATCAGCGTGACTAT; C, GAATTCCTTAACAGGACCCATTTAGGATTAA; D, CTGAGAGT-GGAAGTGTGCGAG; E, GGGATTACAGGCGTG; F, AGACTAACTCAAAT.

structure and drift. It has been predicted on theoretical grounds that the Y specific region and parts closely linked to it should show less polymorphism than the corresponding parts on the X chromosome^{5,6}. Consistent with this, we have found little variation in the boundary region of the Y chromosome. Similarly, other studies have shown low levels of variation in the Y unique regions⁷. The variation in the X chromosomes, on the other hand, is more extensive, but highly structured. At the three central loci, two haplotypes dominate with a third recombinant haplotype only found in some African populations. This may imply that for this region most human X chromosomes descended from only two chromosomes with long independent earlier histories. In any case, the data indicate that the carrier of the chromosome from which all Y boundaries are derived today lived much later than the corresponding carrier of the original X boundary sequence. □

Received 3 October 1989; accepted 30 January 1990.

1. Simmler, M.-C. *et al. Nature* **317**, 692–697 (1985).
2. Ellis, N. A. & Goodfellow, P. N. *Trends Genet.* **5**, 406–410 (1989).
3. Ellis, N. A. *et al. Nature* **337**, 81–84 (1989).
4. Ellis, N., Kidd, J., Goodfellow, P. J., Kidd, K. & Goodfellow, P. N. *Am. J. hum. Genet.* (in the press).
5. Clark, A. G. *Genetics* **115**, 569–577 (1987).
6. Clark, A. G. *Genetics* **119**, 711–720 (1988).
7. Jakubiczka, S., Arremann, J., Cooke, H. J., Kowczak, M. & Schimke, J. *Hum. Genet.* **84**, 86–88 (1989).
8. Li, H. *et al. Nature* **335**, 414–417 (1988).
9. Saiki, R. K. *et al. Science* **230**, 1350–1354 (1986).
10. Wallace, D. *Meth. Enzym.* **152**, 46–47 (1987).
11. Wallace, R. B. & Miyada, C. G. *Meth. Enzym.* **152**, 438–439 (1987).
12. Tabor, S. & Richardson, C. C. *Proc. natn. Acad. Sci. U.S.A.* **84**, 4767–4771 (1987).

ACKNOWLEDGEMENTS. We thank Kenneth Kidd, Jeremy Martinsen and Trefor Jenkins for discussion and for human DNA samples, and Clare Middlemiss for preparing the manuscript.

A frame-shift mutation in the cystic fibrosis gene

Marga B. White*, Jean Amos†, Julie M. C. Hsu‡, Bernard Gerrard*, Paula Finn† & Michael Dean*

* Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland 21701, USA

† Boston University Medical Center, Boston, Massachusetts 21128, USA

‡ Wayne State University School of Medicine, Detroit, Minnesota 48201, USA.

CYSTIC FIBROSIS (CF) is a common recessive lethal genetic disorder, affecting 1 in 1,600 Caucasians¹. The disease causes defective regulation of chloride-ion transport in exocrine cells^{2–5}. Although in all CF families the disease is linked to a locus on chromosome 7q31 (refs 6–11), there is clinical heterogeneity in the severity of the disease and the age at which it is diagnosed. CF is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene^{12–13}. A three-nucleotide deletion ($\Delta F508$) causing the loss of a phenylalanine residue in the tenth exon of the CFTR gene has been found on 70% of CF chromosomes^{12,14}. We have now characterized a CF family in which neither parent of the affected individual carries the common mutation, and identified a two-nucleotide insertion in the CF allele of the mother. The mutation introduces a termination codon in exon 13 of the CFTR gene at residue 821, and is predicted to result in the production of a severely truncated nonfunctional protein.

We identified >70 CF patients having at least one chromosome that did not contain the $\Delta F508$ deletion. To identify additional mutations in these patients we amplified DNA from exons 10, 13, and 17 by the polymerase chain reaction (PCR)¹⁵. The amplified DNA was digested with restriction enzymes to detect insertions, deletions or restriction site changes. No reproducible alterations were detected in exons 10 or 17 when assayed with four and eight enzymes, respectively. In one individual (no. 295 of family BOS22), however, digestion of exon 13

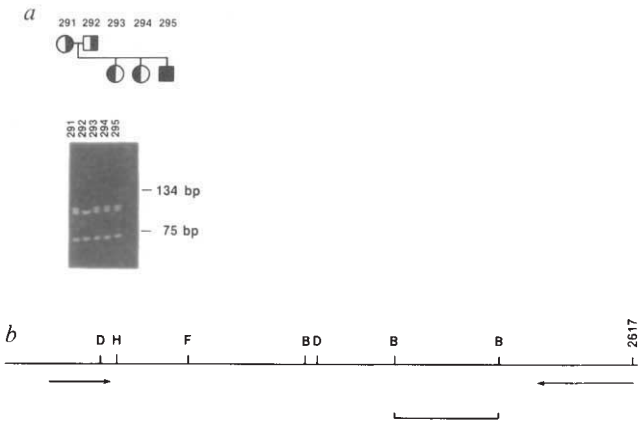


FIG. 1 Localization of a CF mutation in exon 13. *a*, Segregation of the insertion in family BOS22. Top, pedigree with squares representing males and circles representing females. The affected individual is represented by a filled symbol, and carriers are represented by half-filled symbols. Bottom, amplified DNA from each individual digested with *DdeI* and separated on a nondenaturing polyacrylamide gel. Individuals carrying the inserted allele show the presence of a 183/185-bp doublet and higher-mobility fragments corresponding to heteroduplex products. *b*, Map of 3' end of exon 13 of the CFTR gene. Arrows show the location of primers used for PCR amplification, and the line under the sequence denotes the minimum region to which the insertion was localized. Number is nucleotide position. B, *BsrI*; D, *DdeI*; H, *HaeIII*; F, *HinfI*.

METHODS. Amplification of DNA samples (0.1 µg) was performed using primers CF-17 (5'-TGAACCTGATGACACACTCA) and CF-8 (5'-ACAAGCTTGCTCTTCGTTAATTTCTTC) in 25-µl PCR reactions¹⁵. Product (10 µl) was digested with *DdeI* and electrophoresed on a 1.5-mm × 20-cm 8% polyacrylamide gel in TBE buffer²⁶. The mapping experiments were performed similarly, except that 0.1 µl [³²P]dCTP was included in the amplification, and the product was run on 0.4-mm × 40-cm gels. Water controls were consistently blank, and all the sequence matched the reported sequence.

resulted in the production of an extra fragment (data not shown). Restriction mapping demonstrated that this alteration is due to a small insertion in a 31-base pair (bp) *BsrI* fragment near the 3' end of the exon (Fig. 1*b*); the rest of the exon is unaltered (data not shown). Digestion of amplified DNA from this region with *DdeI* followed by PAGE provides a convenient diagnostic assay for the insertion. Lower-mobility products caused by the formation of heteroduplex molecules, consisting of the wild-type and the mutant allele products, were also detected in samples from individual no. 295 and relatives who carried the mutation (Fig. 1*b*). We have termed this new mutant CF allele CFIns2566.

Analysis of the parents and siblings of the proband demonstrated that the mother (no. 291) was a carrier of the insertion-containing allele (Fig. 1*b*, Table 1). The insertion segregates with the mother's CF chromosome, which contains the A (1, 1) haplotype detected by the XV2c and KM19 markers (Table 1).

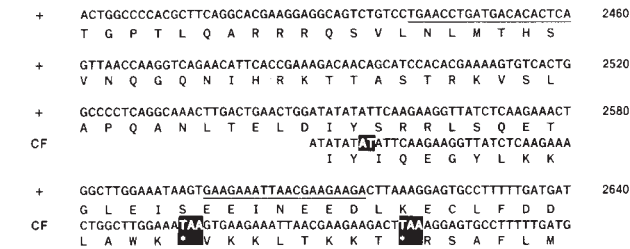


FIG. 2 Sequence of the region surrounding the CFIns2566 mutation and corresponding amino-acid sequence (single-letter code). The sequence for a portion of exon 13 is shown for both normal and mutated alleles; the sequence is numbered as described previously¹². Sequences used to synthesize oligonucleotide primers are underlined. The position of the two-nucleotide insertion and the predicted termination codons are shown in bold.

Although both maternal chromosomes had the A haplotype, other informative markers demonstrated which of these carried the mutant allele (Table 1). Her two daughters (no. 293, no. 294) also carry the insertion, are unaffected by CF, and inherited a different paternal chromosome from that inherited by the affected child. The father (no. 292) is not a carrier of either the ΔF508 deletion or the insertion, and presumably carries a third mutant CF allele on the B (1, 2) haplotype.

Analysis of 75 CF chromosomes without the ΔF508 deletion from other CF patients and obligate carriers did not reveal other families in which this mutation was segregating. (This sample also included five normal chromosomes; data not shown.) It therefore seems that this mutation accounts for <1% of CF chromosomes, although the frequency could be higher in certain population isolates. The BOS22 family is Caucasian and the mother's parents are of Italian and French-Canadian heritage.

Direct DNA sequencing of the normal and mutant alleles revealed that the insertion is two nucleotides long and causes the extension of a short stretch of AT dinucleotides (Fig. 2 and 3). There were no other nucleotide changes in this region that could compensate for the deletion without introducing termination codons (data not shown). This mutation is predicted to shift the normal reading frame and introduce a UAA (ochre) termination codon at residue 821 (Fig. 2).

The CFIns2566 mutation could cause CF by producing either a nonfunctional allele or a mutant allele coding for a protein

TABLE 1 Individual genotypes for pedigree BOS22

		291	292	293	294	295
B79	<i>MspI</i>	1/2	2/1	2/2	2/2	2/1
	<i>HindIII</i>	1/2	2/1	2/2	2/2	2/1
XV		1/1	2/1	1/2	1/2	1/1
KM		1/1	1/2	1/1	1/1	1/2
CF		+/-	+/-	-/+	-/+	-/-
ΔF		+/+	+/+	+/+	+/+	+/+
CFIns		+/-	+/+	-/+	-/+	-/+
W32		1/2	2/1	2/2	2/2	2/1
3.11	<i>MspI</i>	2/2	2/1	2/2	2/2	2/1
	<i>TaqI</i>	1/1	1/1	1/1	1/1	1/1
J29	<i>PvuII</i>	2/2	2/2	2/2	2/2	2/2
	<i>EcoRV</i>	2/2	2/2	2/2	2/2	2/2

Relationships are as shown in Fig. 1. +, Wild-type allele; -, mutant. CF, cystic fibrosis chromosomes; ΔF, 3-bp phenylalanine deletion; CFIns, CF Ins2566 insertion. 1 indicates allele 1 (the larger allele) and 2, allele 2, for each polymorphism. The polymorphic probes used were B79 (D7S13) (ref. 22), XV2c (D7S23) (ref. 23), KM19 (D7S23) (ref. 23), W32 (D7S424) (ref. 24), 3.11 (D7S8) (ref. 25) and J29 (D7S426) (ref. 24). Alleles are arranged as haplotypes, with maternal alleles on the left and paternal alleles on the right.

that has a deleterious effect on exocrine cells. The CFTR gene has been proposed to contain two sets of six transmembrane segments and two ATP-binding domains¹². The predicted CFIns2566 polypeptide would retain its signal sequence, half of the transmembrane segments, and one ATP-binding site. Such a molecule could function aberrantly or associate with normal products and impair their function.

The affected child in family BOS22 was diagnosed as having CF at 2.5 months of age after hospitalization for failure to thrive, and for nutritional and respiratory problems. *Pseudomonas aruginosa* was cultured from the lungs of the patient, but by 8 months he had recovered to an average weight. At 7 years of age, he had a nearly normal chest X-ray pattern (Brasfield score 20/25). Pulmonary function tests revealed mild airway obstruction. Both of the other siblings in this family were carriers and had aberrant levels of chloride ions in their sweat. High sweat-test values are a diagnostic criteria for CF and are not typically elevated in carriers¹⁶⁻¹⁸. The oldest carrier sibling had consistently high sweat-test values (45-67 milliequivalents Cl⁻),

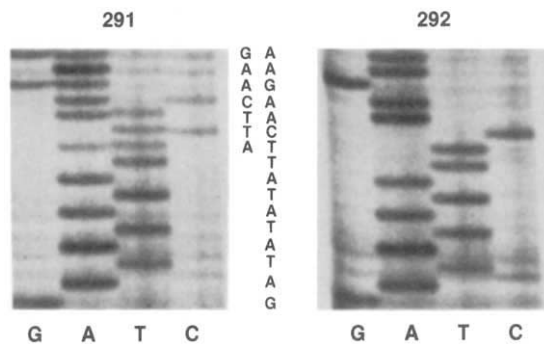


FIG. 3 Direct sequencing of the parents of the affected individual in family BOS22. PCR-amplified DNA was purified and asymmetrically amplified. The resulting single-stranded DNA was sequenced with the CF-17 primer. The sequence for the mother (no. 291) matches that of the father (no. 292) and the published sequence up to the T residue (position 2,566). Beyond this point, the sequence is a mixture of the two alleles owing to an AT insertion. METHODS. DNA amplified with primers CF-8 and CF-17 was electrophoresed on a nondenaturing polyacrylamide gel. The product was excised from the gel and soaked in 100 μ l water for 1 h at 65 °C. Eluted DNA (5 μ l) was reamplified with a 50-fold dilution of primer CF-17 for 40 cycles. The amplified DNA was purified with a Centricon 100 column (Amicon) and sequenced with Sequenase (USB) using dITP and 35 S-labelled dATP.

whereas the other carrier sibling had borderline values at 6–7 years of age (49–50 milliequivalents Cl^-) that decreased to normal levels (38 milliequivalents Cl^-) at 8 years of age. The mother had a normal sweat-test value. The difference in sweat-test values among these individuals could be due to environmental factors or inaccuracies in the sweat test, or reflect additional genetic control over ion transport in the sweat duct. In either case, a more detailed characterization of ion transport and regulation in these individuals should provide insight into these processes.

The CFIns2566 allele is due to the addition of an AT dinucleotide into a short segment (8 bp) of AT dinucleotides. Dinucleotide repeats are hotspots for mutations^{19,20}. Although principally CA repeats have been examined, polymorphic AT repeats have also been characterized. A search of the primate sequences in the computerized database GenBank revealed >50 sequences containing AT repeats that were of 22 bp or more (M.D., data not shown). The mechanism for generating new alleles at these loci is not understood, but could involve unequal crossing-over or errors in replication. Examination of new alleles at other tandemly repeated loci, however, indicates that more complex mechanisms could be involved²¹.

The identification of all of the mutations that cause CF is essential for complete detection and diagnosis of the disease. Although the CFIns2566 allele seems to be rare, the identification of this mutation provides some important insights. First, all of the CF mutations do not lie in the same exon, implying that complete detection will probably require examination of several regions of the gene. Second, frame-shift and other null mutations might not be uncommon. The most likely explanation for the failure so far to find such mutations in the CF gene is that individuals homozygous for the loss of gene function do not survive. If carriers for termination mutants are healthy, there would be no selection against such alleles; these alleles would only appear in CF individuals, however, when balanced by a less severe allele. Frame-shift mutations could occur in virtually any region of the gene, making CF diagnosis difficult.

The continued identification of mutations in the CF locus is expected to help elucidate which regions of the CFTR are functionally important. Also, examination of the effects of these mutations in the allelic combinations in which they naturally occur should greatly increase our understanding of the function of the CFTR gene and its role in disease. □

Received 4 December 1989; accepted 6 February 1990.

1. Boat, T. F., Welsh, M. J. & Beaudet, A. L. in *The Metabolic Basis of Inherited Disease* (eds Scriver, C. L., Beaudet, A. L., Sly, W. S. & Valle, D.) 2649–2680 (McGraw-Hill, New York, 1987).
2. Frizzell, R. A. *Trends Neurosci.* **10**, 190–193 (1987).
3. Jetten, A. M., Yankaskas, J. R., Stutts, M. J., Willumsen, N. J. & Boucher, R. C. *Science* **244**, 1472–1475 (1989).
4. Hwang, T.-C. *et al. Science* **244**, 1351–1353 (1989).
5. Li, M. *et al. Science* **244**, 1353–1356 (1989).
6. White, R. *et al. Nature* **318**, 382–384 (1985).
7. Dean, M. *et al. Nature* **318**, 385–388 (1985).
8. Tsui, L.-C. *et al. Science* **230**, 1054–1057 (1985).
9. Wainwright, B. J. *et al. Nature* **318**, 384–385 (1985).
10. Lathrop, G. M. *et al. Am. J. hum. Genet.* **42**, 38–44 (1988).
11. Dean, M. *Genomics* **3**, 93–99 (1988).
12. Riordan, J. R. *et al. Science* **245**, 1066–1073 (1989).
13. Rommens, J. M. *et al. Science* **245**, 1059–1065 (1989).
14. Kerem, B.-S. *et al. Science* **245**, 1073–1080 (1989).
15. Saiki, R. K. *et al. Science* **239**, 487 (1988).
16. Rosenstein, B. J. & Langbaum, T. S. in *Cystic Fibrosis* (ed. Taussig, L. M.) 85–114 (Thieme-Stratton, New York, 1984).
17. Amos, J. A., Janes, S. R. & Erbe, E. W. *Clin. Invest. Med.* **13**, 1–5 (1989).
18. di Sant'Agnes, P. A. & Powell, G. F. *Ann. N.Y. Acad. Sci.* **93**, 555–599 (1962).
19. Litt, M. & Luty, J. A. *Am. J. hum. Genet.* **44**, 397–401 (1989).
20. Weber, J. L. & May, P. E. *Am. J. hum. Genet.* **44**, 388–396 (1989).
21. Wolff, R. K., Nakamura, Y. & White, R. *Genomics* **3**, 347–351 (1988).
22. Estivill, X., Schmidtke, J., Williamson, R. & Wainwright, B. *Hum. Genet.* **74**, 320–322 (1986).
23. Estivill, X. *et al. Am. J. hum. Genet.* **44**, 704–710 (1989).
24. Iannuzzi, M. C. *et al. Am. J. hum. Genet.* **44**, 695–703 (1989).
25. Bartels, I., Grezeschnick, K.-H., Cooper, D. N. & Schmidke, J. *Am. J. hum. Genet.* **38**, 280–287 (1986).
26. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).

ACKNOWLEDGEMENTS. We thank Janet Buchanan for discussions, and Dean Mann and Anjanette Perry for comments on the manuscript. This study was supported by the Department of Health and Human Services.

Biological properties of a CD4 immunoadhesin

Randal A. Byrn*, Joyce Mordenti, Catherine Lucas, Douglas Smith, Scot A. Marsters, Jennifer S. Johnson*, Paul Cossum, Steven M. Chamow, Florian M. Wurm, Timothy Gregory, Jerome E. Groopman* & Daniel J. Capon

Genentech Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA

* Division of Hematology-Oncology, Harvard Medical School, New England Deaconess Hospital, Boston, Massachusetts 02215, USA

MOLECULAR fusions of CD4, the receptor for human immunodeficiency virus (HIV; refs 1–4), with immunoglobulin (termed CD4 immunoadhesins) possess both the gp120-binding and HIV-blocking properties of recombinant soluble CD4, and certain properties of IgG, notably long plasma half-life and Fc receptor binding^{5,6}. Here we show that a CD4 immunoadhesin can mediate antibody-dependent cell-mediated cytotoxicity (ADCC) towards HIV-infected cells, although, unlike natural anti-gp120 antibodies, it does not allow ADCC towards uninfected CD4-expressing cells that have bound soluble gp120 to the CD4 on their surface. In addition, CD4 immunoadhesin, like natural IgG molecules, is efficiently transferred across the placenta of a primate. These observations have implications for the therapeutic application of CD4 immunoadhesins, particularly in the area of perinatal transmission of HIV infection.

We have previously described CD4 immunoadhesins containing the first two immunoglobulin-like domains of CD4 joined to the entire constant region of human IgG1 heavy chain⁵. As the presence of light chain was found to be unnecessary for secretion of dimeric molecules⁵, we constructed additional derivatives lacking the CH1 domain of the IgG1 heavy chain (Fig. 1). The gp120-binding and Fc receptor-binding properties and the improved half-life characteristics of this molecule were comparable to the CD4 immunoadhesin containing the CH1 domain (not shown).

As CD4 immunoadhesin binds Fc receptors, we examined

TABLE 1 Placental transfer of rCD4 and CD4-IgG in pregnant rhesus monkeys

Rhesus monkey	Protein	Concentration (ng ml ⁻¹) in maternal serum		Concentration (ng ml ⁻¹) in newborn serum	Infant/maternal concentration ratio
		Mean	Range		
1	CD4-IgG	489	(276–673)	15.2	3.1%
2	CD4-IgG	217	(155–301)	7.6	3.5%
3	rCD4	682	(360–820)	1.1	0.16%
4	rCD4	437	(205–504)	<0.8	<0.18%

Four pregnant rhesus monkeys at 150–160 days gestation (normal gestation period 160–170 days) received a loading dose of CD4 immunoconjugate (CD4-IgG) or rCD4 by rapid intravenous injection followed by continuous infusion for 24 h; the infants were delivered by caesarian section. Blood was obtained from the mother after 1 min and after 4, 8, 12, 16, 20 and 24 h of infusion and from the infant and cord blood at the time of delivery. For maternal dosing and blood sampling, catheters were placed into a femoral vein and artery under general anaesthesia 24 h before the start of the study. After catheterization the animals were placed into jackets, and no additional anaesthesia was given. Animals received the loading dose of drug as an intravenous bolus into the femoral vein catheter over 5 s followed by saline flush to clear the catheter of drug. The infusion was started immediately thereafter. For CD4 immunoconjugate, a 0.135 mg kg⁻¹ loading dose was given, followed by 1.12 mg kg⁻¹ over 24 h; for rCD4, a 0.135 mg kg⁻¹ loading dose was given, followed by 28 mg kg⁻¹ over 24 h. Serum concentrations of each protein were determined by double antibody enzyme-linked immunosorbent assays (ELISA) each using monoclonal antibody Leu3a (Becton-Dickinson). As this antibody recognizes the gp120 binding domain of CD4, the assays thus detect CD4-containing molecules still capable of binding gp120. To measure rCD4 concentration, Leu3a in 0.05 M carbonate buffer, pH 9.6, was used to coat 96-well microtitre plates overnight at 4 °C. After three washes with PBS containing 0.05% Tween 20 (PBS-Tween), plates were blocked for 1 h at room temperature with ELISA diluent (PBS containing 0.5% BSA, 0.05% Tween 20 and 0.01% thimerosal). rCD4 standards and samples diluted in rhesus serum were incubated for 2 h, and plates were washed again with PBS-Tween. For detection of rCD4, monoclonal antibody OKT4 (Ortho) was conjugated to horseradish peroxidase (HRP, Boehringer Mannheim) by the periodate method¹⁹. After appropriate dilution in ELISA diluent, the conjugated antibody was incubated for 1 h at ambient temperature. Orthophenylene diamine dihydrochloride (Sigma), 2.2 mM in 0.05 M sodium phosphate/0.1 M citrate buffer, pH 5.0, containing 0.01% H₂O₂, was used as a substrate for 20–30 min at room temperature. Reactions were stopped with 4.5 N H₂SO₄ and plates were read at 492 nm. Data were reduced using a four-parameter curve-fitting program²⁰. The range for this assay was 0.8 to 25 ng ml⁻¹. For the measurement of CD4 immunoconjugate, the same procedure was used, except that Leu3a was conjugated to HRP and used for detection; monoclonal antibody L104.5 (provided by B. Fendly, Genentech), which recognizes domain 2 of rCD4, was used for antigen capture. The range for this assay was 0.19 to 12.0 ng ml⁻¹.

whether it could mediate ADCC towards HIV-infected cells by human peripheral blood mononuclear cells. Indeed, CD4 immunoconjugate mediates ADCC towards HIV-infected, but not uninfected, CEM human T-lymphoblastoid cells in a dose-dependent manner (Fig. 2a and b). Soluble recombinant (rCD4) does not mediate ADCC (not shown), but can inhibit cell lysis mediated by CD4 immunoconjugate (Fig. 2a), demonstrating that specific binding to gp120 by CD4 immunoconjugate is essential.

It has been suggested that ADCC in AIDS patients may be a mechanism of pathogenesis rather than protection⁷, as soluble gp120, by binding to healthy CD4-expressing 'bystander' cells, can make such cells targets for ADCC, mediated by the anti-gp120 antibodies found in HIV-infected individuals. In contrast to natural anti-gp120 antibodies, CD4 immunoconjugate does not

mediate killing of uninfected CEM cells preincubated with soluble gp120 (Fig. 2b). A likely explanation is that CD4 immunoconjugate, unlike natural anti-gp120 antibodies, cannot bind gp120 already bound to cell-surface CD4, because soluble gp120 is thought to have only one CD4-binding site.

An increasing number of paediatric AIDS patients are infected *in utero* by transmission from the mother⁸. As natural IgG molecules are selectively transported across the placenta of primates in an Fc receptor-dependent manner, we tested whether CD4 immunoconjugate shared this property. Pregnant rhesus monkeys near to term were given a bolus dose of either rCD4 or CD4 immunoconjugate, then were continuously infused to a relatively constant concentration for 24 h before delivery by caesarian section. Serum concentrations were determined by

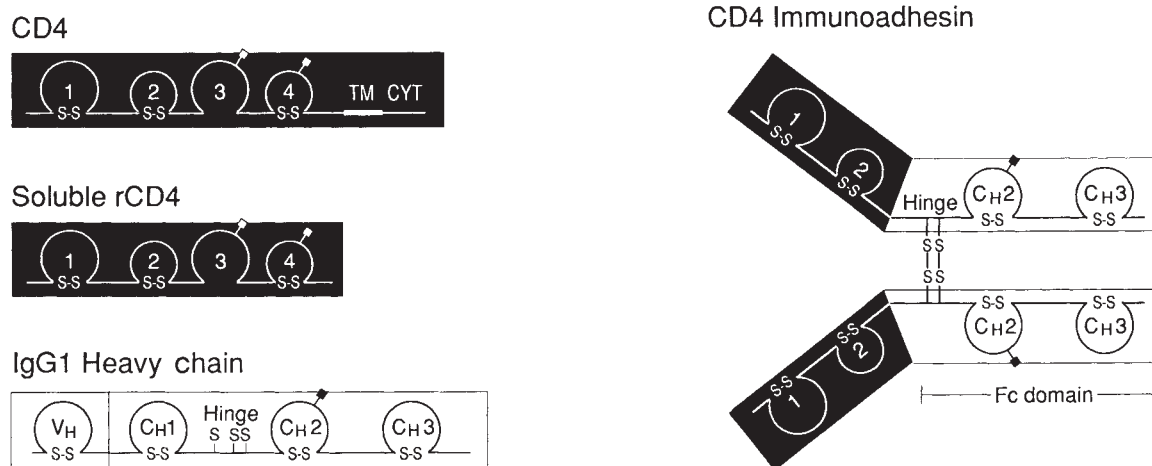


FIG. 1 Structure of CD4 immunoconjugate, soluble rCD4 and the parent human CD4 and IgG1 heavy chain molecules. CD4- and IgG1-derived sequences are indicated by shaded and unshaded regions, respectively. The immunoglobulin-like domains of CD4 are numbered 1–4; TM and CYT refer to the transmembrane and cytoplasmic domains. Soluble rCD4 is truncated after proline 368 of the mature CD4 polypeptide¹⁴. The variable (VH) and constant (CH1, hinge, CH2, and CH3) regions of IgG1 heavy chain are shown. Disulphide bonds are indicated by S–S. CD4 immunoconjugate consists of

residues 1–180 of the mature CD4 protein fused to IgG1 sequences beginning at aspartic acid 216 (taking amino acid 114 as the first residue of the heavy chain constant region¹⁵) which is the first residue in the IgG1 hinge after the cysteine residue involved in heavy-light chain bonding. The CD4 immunoconjugate shown, which lacks a CH1 domain, was derived from a CH1-containing CD4 immunoconjugate⁵ by oligonucleotide-directed deletional mutagenesis¹⁶, expressed in Chinese hamster ovary cells and purified to >99% purity using protein A-Sepharose chromatography as described⁵.

immunoassay at various times in the mother and in the newborn within 5 min of birth. The concentration of CD4 immunoadhesin in fetal serum was $\geq 3\%$ of the maternal level after 24 h (Table 1), indicating a significant rate of placental transfer. By contrast, rCD4 did not accumulate in the fetal serum to a significant extent. This is most probably due to lack of active transport across the placental barrier, although it is possible that transfer would not be detected owing to the shorter half-life of rCD4 (ref. 5).

Although the rate at which a protein appears in the fetal circulation cannot be directly translated into a rate of placental transfer, because the rate of degradation of the protein in the fetus is unknown, comparisons can be made with the appearance rate of human antibody in classical human experiments. Dancis *et al.*⁹ gave radioiodinated human γ -globulin to women in their third month of pregnancy before abortion of the fetus, and observed a concentration in the fetus that was 2.8% of maternal levels after 18–24 h. Similarly, Gitlin *et al.*¹⁰ gave women who were nearly to term a single intravenous injection of radioiodinated γ -globulin up to 4 weeks before birth and observed an increase in the infant plasma concentration of $\sim 3\%$ of maternal level per day. Thus the rate of appearance of CD4 immunoadhesin in a primate fetus is close to that of normal human IgG in humans.

CD4-based strategies have an important theoretical advantage

over other AIDS therapeutics, as HIV must bind CD4 to be able to infect its cellular target (the T4 cell) specifically. Soluble CD4 derivatives have thus been developed with two objectives: to block gp120-mediated events such as the spread of viral infection, formation of syncytia and binding of gp120 to uninfected 'bystander' cells, and to use CD4 as a targeting agent to direct a cytotoxic agent to HIV-infected cells (for example, CD4-ricin¹¹, CD4-pseudomonas exotoxin¹²). Here we have shown that CD4 immunoadhesin can direct the killing of HIV-infected cells, as well as blocking gp120-mediated events^{5,6}. Significantly, CD4 immunoadhesin, unlike natural anti-gp120 antibodies, cannot kill CD4-expressing bystander cells coated with soluble gp120.

The fetal acquisition of passive immunity in humans is mediated by selective placental transfer of maternal IgG. As CD4 immunoadhesin shares this property, passive immunity to HIV could be established in the fetus by maternal administration, possibly preventing perinatal transmission of infection. The mechanism underlying selective transport of IgG involves binding to Fc receptors on the apical surface of the syncytiotrophoblast, resulting in protected endocytotic transport¹³. The fact that this, and so many other different properties of IgG, can be conferred on CD4 by the addition of an Fc region suggests that such functions could be acquired by any adhesion molecule capable of being linked to Fc in place of the Fab sequences

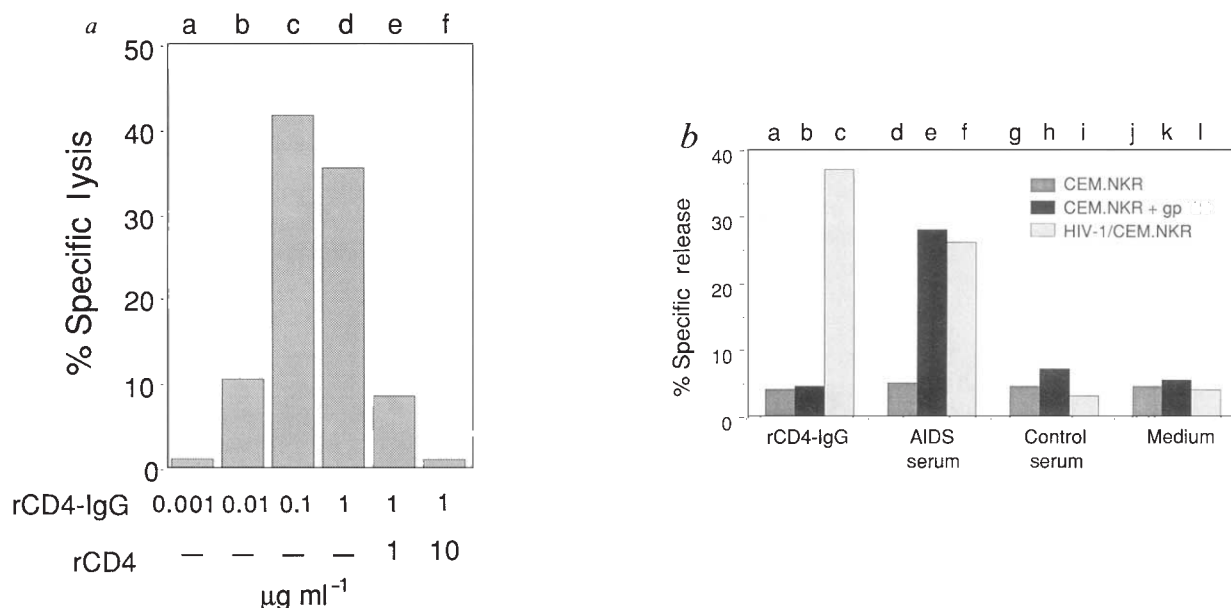


FIG. 2 Antibody-dependent cell-mediated cytotoxicity (ADCC) shown by CD4 immunoadhesin. CEM T-lymphoblastoid target cells were labelled with ^{51}Cr , incubated with CD4 immunoadhesin, rCD4, serum, or control media for 30 min, and mixed with peripheral blood mononuclear cells (PBMC), as effector cells, at an effector-to-target ratio of 50:1. The cell mixtures were incubated for 20 h at 37°C , and the cell-free supernatant was collected and assayed for ^{51}Cr released from target cells. **a**, Lysis of HIV-1-infected CEM.NKR target cells by effector cells in the presence of CD4 immunoadhesin at 0.001 (lane a), 0.01 (lane b), 0.1 (lane c) and $1.0 \mu\text{g ml}^{-1}$ (lanes d–f). Also shown is the blocking by rCD4 at $1.0 \mu\text{g ml}^{-1}$ (lane e) and $10 \mu\text{g ml}^{-1}$ (lane f) of target cell lysis mediated by $1.0 \mu\text{g ml}^{-1}$ CD4 immunoadhesin. The level of cell lysis observed with CD4 immunoadhesin was comparable to that mediated by a control AIDS patient serum. rCD4 itself does not mediate target cell lysis at concentrations up to $10 \mu\text{g ml}^{-1}$. Uninfected CEM.NKR targets were not lysed by effector cells in the presence of CD4 immunoadhesin, AIDS patient serum or normal human serum (see below), but could be lysed in the presence of a rabbit anti-rCD4 serum (not shown). **b**, ADCC towards uninfected CEM.NKR target cells (lanes a, d, g and j), uninfected CEM.NKR cells incubated with soluble gp120 (ref. 17) (lanes b, e, h and k) and HIV-1-infected CEM.NKR (lanes c, f, i and l) mediated by CD4 immunoadhesin (lanes a–c), AIDS patient serum at 1/1,000 final dilution (lanes d–f), serum from an uninfected individual at 1/1,000 final dilution (g–i) and complete medium (lanes j–l).

METHODS. The CEM.NKR T-lymphoblastoid cell line, which is resistant to NK-mediated lysis¹⁸, was used for all experiments. HIV-1-infected CEM.NKR cells were produced by inoculating 10^6 CEM.NKR cells with 10^3 TCID₅₀ of HIV-1 II_B. The culture was monitored for infection using reverse transcriptase (RT) activity and HIV-1 specific antibodies for immunofluorescence. After ~ 2 weeks the culture became stable, with $> 70\%$ of cells

immunofluorescence-positive, and $> 10^6$ c.p.m. ml^{-1} RT activity in the medium. Cells were maintained in RPMI 1640 medium (Gibco) containing 20% fetal bovine serum (MA bioproducts), penicillin, streptomycin and L-glutamine (complete medium). Target cells were labelled by incubation of 10^6 cells with $100 \mu\text{Ci } ^{51}\text{Cr}$ in 0.5 ml for 2 h at 37°C . After two washes, cells were suspended at 2×10^5 per ml in complete medium and 25- μl aliquots (containing 5×10^3 cells) were dispensed to wells of a 96-well plate. For the lysis assay, 25 μl purified recombinant proteins or sera diluted in complete medium, or control medium, were added to each well and incubated for 30 min at room temperature. Assays were carried out in triplicate. Effector PBMCs were prepared from heparinized blood obtained from a healthy, HIV-seronegative donor by centrifugation through Ficoll-Paque (Pharmacia). After two washes in RPMI 1640 the cells were suspended to 5×10^6 cells per ml in complete medium and 50- μl aliquots were added to appropriate wells. The total incubation volume was therefore 100 μl . The concentrations indicated are final concentrations after effector cells were added. Plates were incubated for 16–18 h at 37°C in 5% CO_2 . For analysis of cell lysis, 50- μl samples of supernatant were pipetted from each well, mixed with detergent to inactivate HIV, then mixed with 0.5 ml Protosol (New England Nuclear) and 5 ml Betafluor (National Diagnostics) and analysed by scintillation counting. Maximum lysis, spontaneous lysis and complete medium controls were included in each assay for each target cell, in triplicate. Maximum lysis was obtained by substituting 25 μl of 2% Triton X-100 for the test sample. Spontaneous release wells received 70 μl complete medium instead of effector cells. Complete medium controls received medium instead of the test sample. Percentage specific lysis was calculated using the formula, % specific lysis = (test sample – spontaneous release)/(maximum lysis – spontaneous release).

normally constituting the antigen-binding site of IgG. Therefore, in principle, any such receptor can be given the functional characteristics of an antibody, with the ability to select desirable characteristics at will. □

Received 7 December 1989; accepted 19 February 1990.

1. Dalglish, A. *et al. Nature* **312**, 763–767 (1984).
2. Klatzmann, D. *et al. Nature* **312**, 767–768 (1984).
3. Maddon, P. *et al. Cell* **47**, 333–348 (1986).
4. McDougal, J. *et al. Science* **231**, 382–385 (1986).
5. Capon, D. *et al. Nature* **337**, 525–531 (1989).
6. Traunecker, A., Schneider, J., Kiefer, H. & Karjalainen, K. *Nature* **339**, 68–70 (1989).
7. Lyerly, H., Matthews, T., Langlois, A., Bolognesi, D. & Weinhold, K. *Proc. natn. Acad. Sci. U.S.A.* **84**, 4601–4605 (1987).
8. Katz, S. & Wilfert, C. *New Engl. J. Med.* **320**, 1687–1688 (1989).
9. Dancis, J., Lind, J., Oratz, M., Smolens, J. & Vara, P. *Am. J. Obstet. Gynec.* **82**, 167–171 (1961).
10. Gitlin, D., Kumate, J., Urrusti, J. & Morales, C. *J. clin. Invest.* **43**, 1938–1951 (1964).
11. Till, M. *et al. Science* **242**, 1166–1168 (1988).
12. Chaudhary, A. *et al. Nature* **335**, 369–372 (1988).
13. Johnson, P. & Brown, P. *Placenta* **2**, 355–369 (1981).
14. Smith, D. *et al. Science* **238**, 1704–1707 (1987).
15. Kabat, E. *et al. Sequences of Proteins of Immunological Interest* 4th edn (1987).
16. Zoller, M. & Smith, M. *Nucleic Acids Res.* **10**, 6487–6500 (1982).
17. Lasky, L. *et al. Science* **233**, 209–212 (1986).
18. Howell, D., Andriotti, P., Dawson, J. & Cresswell, P. *J. Immun.* **134**, 971 (1985).
19. Nakane, P. & Pierce, G. *J. Cell Biol.* **33**, 307–318 (1967).
20. Marquardt, D. *J. Soc. ind. appl. Math.* **11**, 431–441 (1963).

ACKNOWLEDGEMENTS. We thank Steven Frie for performing enzyme-linked immunosorbent assays, Dr Pam Frost and Natalie Gaylord (Primate Research Institute, New Mexico State University) for assistance with animal studies, and Dr Rebecca Ward for critical comments on the manuscript. This work was supported by Genentech, Inc. and the NIH (R.B. and J.G.)

Calcium entry through stretch-inactivated ion channels in *mdx* myotubes

Alfredo Franco Jr & Jeffry B. Lansman*

Department of Pharmacology, School of Medicine, University of California, San Francisco, California 94143-0450, USA

RECENT advances in understanding the molecular basis of human X-linked muscular dystrophies (for a review, see ref. 1) have come from the identification of dystrophin, a cytoskeletal protein associated with the surface membrane^{2–4}. Although there is little or virtually no dystrophin in affected individuals^{5,6}, it is not known how this causes muscle degeneration. One possibility is that the membrane of dystrophic muscle is weakened and becomes leaky to Ca^{2+} (refs 7–9). In muscle from *mdx* mice, an animal model of the human disease¹⁰, intracellular Ca^{2+} is elevated and associated with a high rate of protein degradation¹¹. The possibility that a lack of dystrophin alters the resting permeability of skeletal muscle to Ca^{2+} prompted us to compare Ca^{2+} -permeable ionic channels in muscle cells from normal and *mdx* mice. We now show that recordings of single-channel activity from *mdx* myotubes are dominated by the presence of Ca^{2+} -permeable mechano-transducing ion channels. Like similar channels in normal skeletal muscle, they are rarely open at rest, but open when the membrane is stretched by applying suction to the electrode^{12–14}. Other channels in *mdx* myotubes, however, are often open for extended periods of time at rest and close when suction is applied to the electrode. The results show a novel type of mechano-transducing ion channel in *mdx* myotubes that could provide a pathway for Ca^{2+} to leak into the cell.

We recorded single-channel activity from cell-attached patches on myotubes from normal and *mdx* mice with 110 mM BaCl_2 in the patch electrode. Figure 1a shows a continuous record of single-channel activity recorded ~1 min after the patch electrode formed a seal on the surface of a myotube from normal mouse muscle. At a holding potential of –60 mV, the single-channel

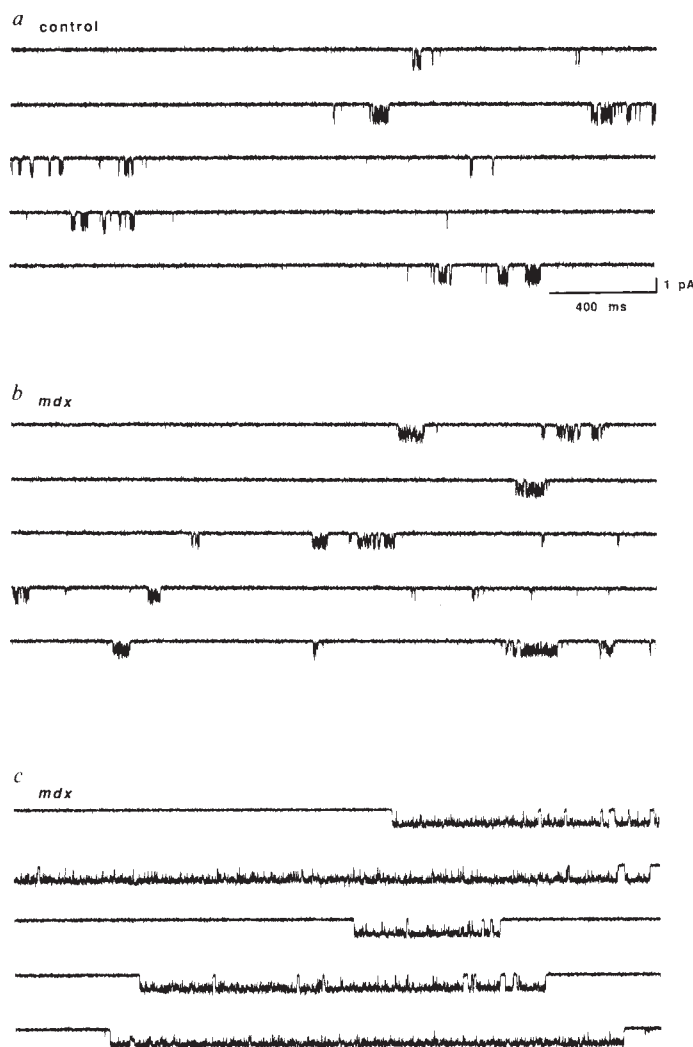


FIG. 1 Channel activity recorded from the surface of myotubes from normal and *mdx* mice with 110 mM BaCl_2 in the patch electrode showing unitary Ba^{2+} currents at a constant holding potential of –60 mV. The traces are sequential and represent a segment of a continuous recording (~10 seconds channel activity). Currents were filtered at 1 kHz with an eight-pole Bessel filter and sampled at 5 kHz. *a*, Recording from a cell-attached patch on a normal myotube. *b*, Recording from a cell-attached patch on a *mdx* myotube showing low channel activity. *c*, Recording from a different *mdx* myotube in which channel activity was high.

METHODS. Myotubes were prepared by dissecting hind-limb or cutaneous-pectoris muscles from 7-day-old normal C57B control mice or *mdx* mice (Jackson Laboratory) after killing by cervical dislocation. The muscle was minced and incubated for ~15 min at 37 °C in Ca^{2+} - and Mg^{2+} -free Hank's buffer containing 0.125% trypsin. Cells were dissociated by passing through a small-bore pipette and filtered through 100- μm gauze. The suspension was preplated for ~1 h to remove fibroblasts, after which the remaining cells in suspension were plated on gelatin-coated tissue culture dishes at a density of ~5,000 cells per cm^2 in DMEM medium supplemented with 20% FCS and chick embryo extract. Myoblasts began to fuse and form myotubes after ~4–5 days in culture. Recordings were made from myotubes 1–5 days after the first myotubes formed. Recordings of single-channel activity from cell-attached patches were made with a List EPC-7 amplifier as described previously¹⁹. Current signals were recorded on video tape and replayed onto the hard disk of a laboratory computer (PDP 11/73) for later analysis. Patch electrodes were made from borosilicate capillary pipettes (Rochester Scientific) and had resistances of 2–4 M Ω when filled with 110 mM BaCl_2 and immersed in the bath. The bathing solution contained 150 mM potassium aspartate, 5 mM MgCl_2 , 5 mM EGTA, 10 mM glucose and 10 mM HEPES buffer. The pH was adjusted to 6.5 with KOH. An isotonic potassium bathing solution was used to zero the resting potential of the cell. Occasionally, voltage shifts were detected after patch excision which indicated a maximum voltage error of ~10 mV. The bathing solution produced no obvious signs of cell deterioration.

* To whom correspondence should be addressed.

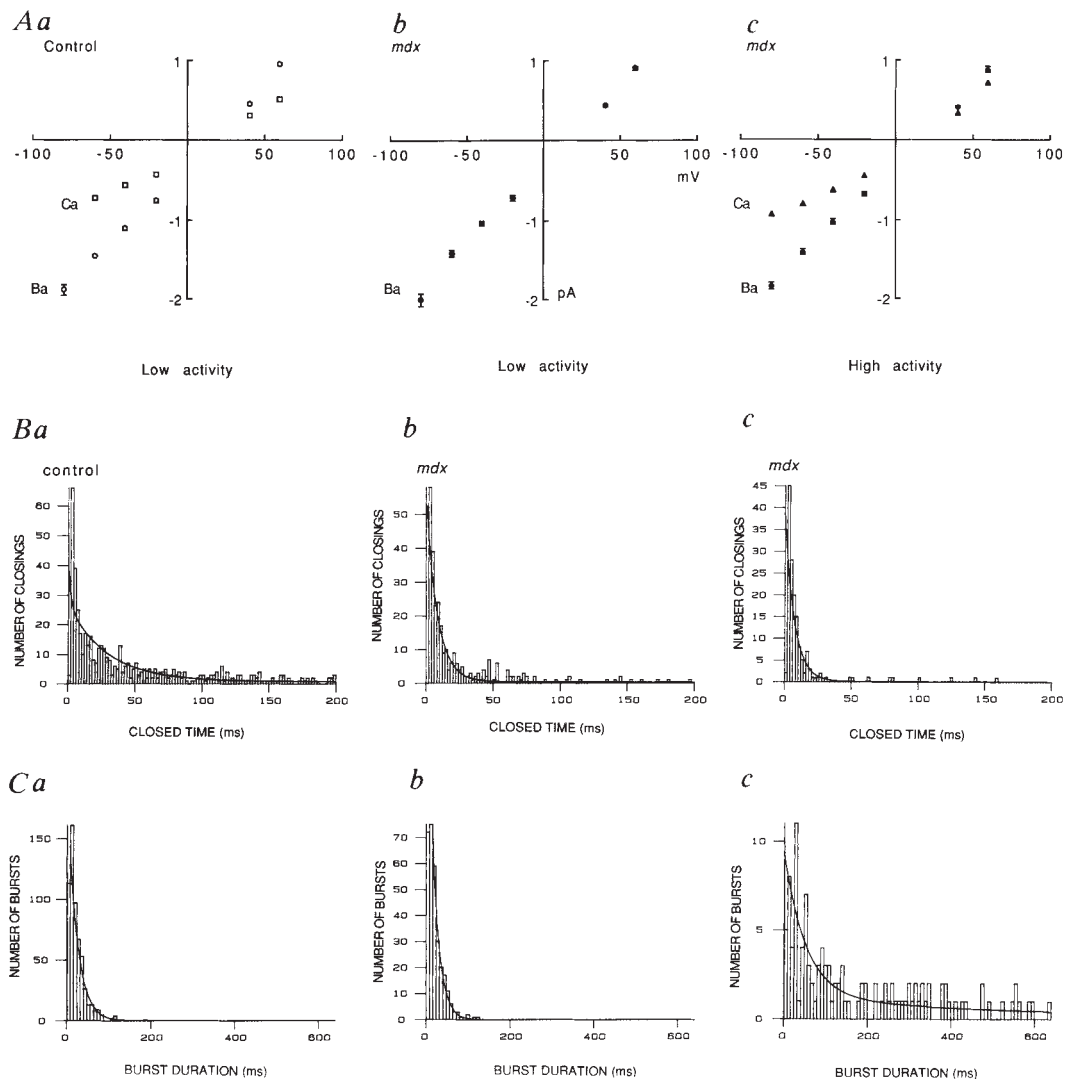
currents carried by Ba^{2+} appeared as discrete steps of ~ 1.5 pA. A plot of the amplitude of the unitary Ba^{2+} current as a function of the patch potential is shown in Fig. 2Aa (open circles). The relationship is linear with a slope conductance of 17.5 pS. Channel events could also be detected with 110 mM CaCl_2 in the electrode, although they were smaller (~ 1.0 pA at -60 mV) and had a slope conductance of ~ 8 pS (open squares). The current-voltage (i - V) relationships show that in the presence of either Ba^{2+} or Ca^{2+} the current becomes positive above 0 mV, but below the equilibrium potential for Ca^{2+} , indicating the channel is more permeable to divalent than to monovalent cations. The relative permeability of Ca^{2+} to K^+ , the main intracellular cation, is $P_{\text{Ca}^{2+}}/P_{\text{K}^+} = \sim 5$.

Figure 1b, c shows two different recordings from cell-attached patches on the surface of *mdx* myotubes. In many recordings from *mdx* myotubes, single-channel activity was low and similar to that recorded from normal myotubes (Fig. 1b). The single-channel i - V relationships in the presence of 110 mM Ba^{2+} were similar to those recorded from normal myotubes (Fig. 2A, compare a and b). Roughly 30% of the recordings from cell-attached patches on *mdx* myotubes, however, showed very high channel activity (Fig. 1c). The single-channel i - V relationships for high activity recorded from *mdx* myotubes such as that

shown in Fig. 1c are plotted in Fig. 2Ac. The single-channel conductance and reversal potentials measured in the presence of 110 mM BaCl_2 and CaCl_2 were 18 pS and $+18.0$ mV, and 8.5 pS and $+32.0$ mV, respectively. These can be compared with those for the low-activity in normal myotubes, which were 17.5 pS and $+23$ mV and 8 pS and $+38$ mV in the presence of Ba^{2+} and Ca^{2+} , respectively. The results indicate that the low activity channels in normal myotubes and the high activity channels in *mdx* myotubes have very similar permeabilities to divalent cations.

We analysed channel closed times and burst durations to compare channel gating for the channel activity recorded from *mdx* myotubes with that recorded from normal myotubes. Figures 2B and 2C show the results of the kinetic analysis for activity recorded at a holding potential of -60 mV. The distribution of closed times was best fit as the sum of three exponential components with roughly similar time constants (for details see the legend to Fig. 2). The distribution of channel burst durations for the low activity in both normal and *mdx* myotubes (Fig. 2C, a and b) were best fit with a single rapidly decaying exponential component, whereas that for the high activity in *mdx* myotubes (Fig. 2Cc) was better fit as the sum of two more-slowly decaying exponential components, reflecting the longer openings in the

FIG. 2 Comparison of channel activity recorded from normal and *mdx* myotubes. A, Effect of patch holding potential on the amplitude of unitary Ba^{2+} currents recorded from normal (a) and from *mdx* myotubes (b, low activity; c, high activity). Single-channel i - V relationship obtained with electrodes filled with 110 mM Ca^{2+} is also shown. The single-channel i - V relationship gave slope conductances and reversal potentials of 17.5 pS and $+23$ mV ($n=5$) and 8 pS and $+38$ mV ($n=2$) for low channel activity in normal myotubes in the presence of 110 mM Ba^{2+} and Ca^{2+} , respectively. In *mdx* myotubes, the i - V relationship for the low activity (b) gave a conductance and reversal potential in the presence of 110 mM Ba^{2+} of 17.0 pS and $+23.0$ mV ($n=5$); the i - V relationships in the presence of Ba^{2+} and Ca^{2+} for the high activity (c) gave conductances and reversal potentials of 18.0 pS and $+18.0$ mV ($n=9$) and 8.5 pS and $+32.0$ mV ($n=2$), respectively. B and C, comparison of channel kinetics for the three forms of channel activity recorded at a constant holding potential of -60 mV. For the analysis, currents were filtered at 100 Hz to observe the better-resolved slower gating transitions. Histograms of closed times (B) and burst durations (C) for activity recorded from normal (Ba and Ca) and *mdx* myotubes (low activity, Bb and Cb; high activity, Bc and Cc). The smooth curves drawn through the histograms represent the maximum likelihood fit to three exponentials for closed times for normal (Ba; $\tau_1 =$



1.7 ms, $\tau_2 = 29$ ms and $\tau_3 = 491$ ms), low *mdx* activity (Bb, $\tau_1 = 1.3$ ms, $\tau_2 = 9$ ms, and $\tau_3 = 692$ ms), and high *mdx* activity (Bc, $\tau_1 = 1.3$ ms, $\tau_2 = 7.3$ ms, and $\tau_3 = 1168$ ms). C, Histograms of burst durations for normal (Ca, $\tau_1 = 22$ ms), low *mdx* activity (Cb, $\tau = 17$ ms), and high *mdx* activity (Cc, $\tau_1 = 53$ ms and $\tau_2 = 587$ ms).

single-channel records. The results indicate that the low channel activity in normal and *mdx* muscle have similar gating properties.

In recordings from both normal and *mdx* myotubes in which resting activity was low (Fig. 1a, b), applying suction to the electrode often increased channel opening, as expected for the behaviour of stretch-activated channels^{12–14}. To test the possibility that the unusually high channel activity recorded from *mdx* myotubes (Fig. 1c) arises from mechano-transducing ion channels, we applied suction to the patch electrode while recording at a constant holding potential. Surprisingly, applying suction to the electrode decreased, rather than increased, channel activity. A reduction in the activity of mechano-transducing channels with increased suction has previously been reported in invertebrate neurons¹⁵, but not in vertebrate skeletal muscle in which only channels that are opened by suction have been observed^{12–14}.

The records in Fig. 3a show the response to suction applied to the electrode in a recording from an *mdx* myotube in which resting channel open probability was high. There were at least three channels in the patch as indicated by the maximum number of superimposed unitary current steps (opening levels shown at the right of the top record). The top record is the control activity without applied pressure. Applying suction pressure of -20 mm Hg to the electrode reduced channel opening by more than half.

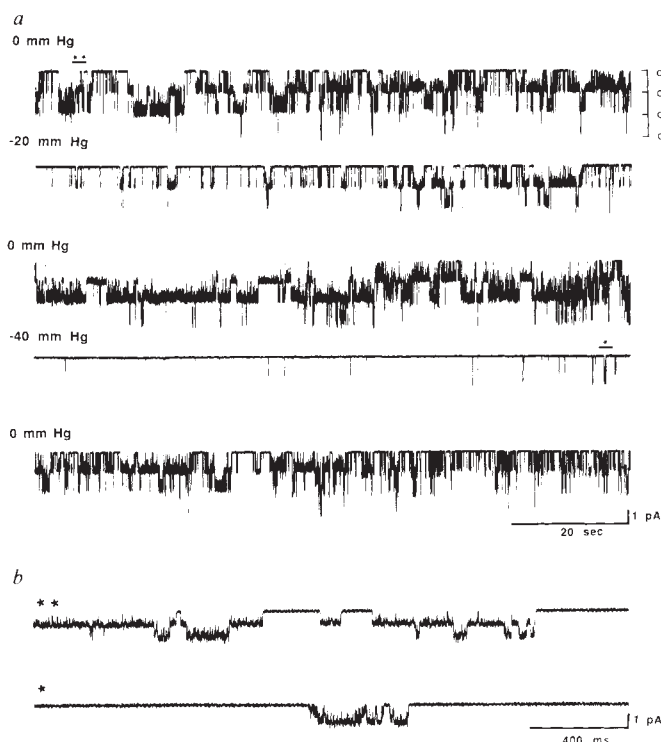


FIG. 3 Effects of suction applied to the patch electrode on the channel activity recorded at a constant holding potential. *a*, Records of single-channel activity recorded from the surface of an *mdx* myotube. The records were taken in sequence. Each trace represents a continuous record of channel activity lasting ~ 100 s. Currents were filtered at 100 Hz and sampled at 1 kHz. The pressure applied to the patch electrode is indicated in mm Hg above each record. Channel open probability was 30% (0 mm, top record), 10% (-20 mm, second-from-top record), 43% (0 mm, middle record), 0.4% (-40 mm, second-from-bottom record) and 16% (0 mm, bottom record). *b*, Records marked with double (control) and single (-40 mm Hg) asterisks plotted on an expanded time scale. Records in *a* are filtered at 100 Hz, whereas the expanded records in *b* are filtered at 1 kHz. The unitary amplitude varied in some cases by ~ 0.3 pA. Although this variation could represent the contribution of channels with slightly different unitary conductances in the multichannel patch, this variability is within the range expected for the current fluctuations in the open channel noise as well as for artefacts arising from slight variations in patch potential.

After releasing the pressure, channel activity once again returned to high levels. Applying a suction pressure of -40 mm Hg to the electrode virtually suppressed channel activity, although very brief openings with same unitary amplitude as the openings observed without applied pressure could still be observed (Fig. 3b, expansion of records marked by asterisks in 3a). Releasing

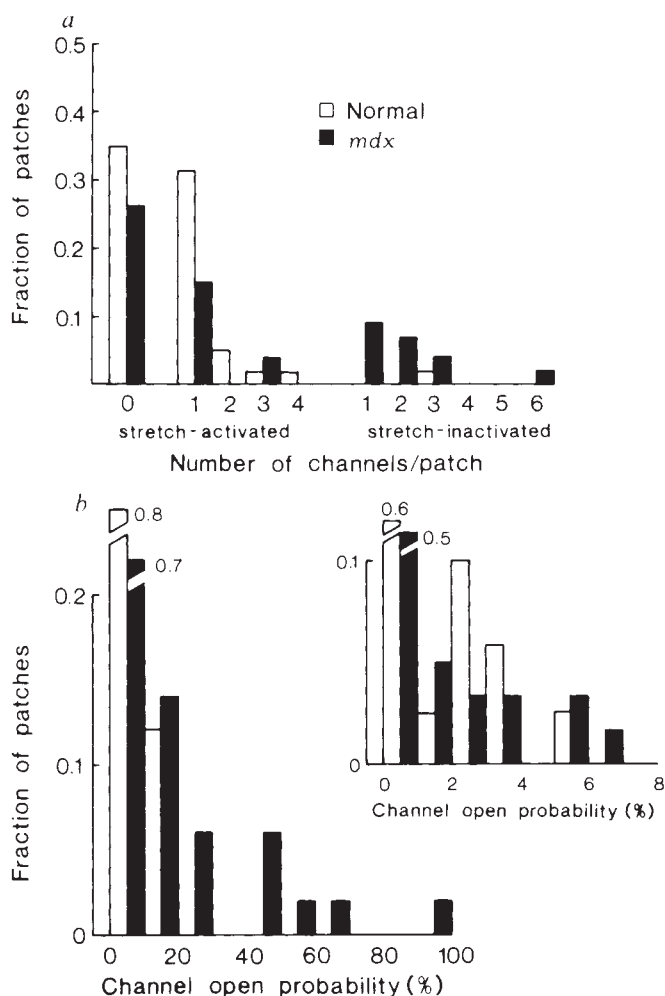


FIG. 4 Comparison of recordings of channel activity from cell-attached patches on normal (open bars) and *mdx* (filled bars) myotubes. The data represent samplings from two separate sets of cultures prepared from normal and *mdx* muscle as described above. *a*, Histogram showing the fraction of all patches without channel activity (no channels) and with n stretch-activated ($n=1-4$) or stretch-inactivated ($n=1-6$) channels. Total number of patches was 43 for normal myotubes and 57 for *mdx* myotubes. The number of channels in a patch was taken as the maximum number of simultaneous openings observed in the single-channel record. Spontaneous activity that was insensitive to suction applied to the electrode¹⁴ is not shown, but accounted for 21% of the patches on normal myotubes and 33% of the patches on *mdx* myotubes. Stretch-activated channels were observed in 42% of the patches on normal myotubes and 19% of the patches on *mdx* myotubes; stretch-inactivated channels were observed in 2% of the patches on normal myotubes and 21% of the patches on *mdx* myotubes. The number of stretch-inactivated channels observed could be an underestimate if the suction applied to the patch membrane during seal formation deformed the patch sufficiently so that channels were inactivated during the experiment. *b*, Histogram comparing mean channel open probability recorded from cell-attached patches on normal (30 patches) and *mdx* (51 patches) myotubes. Channel open probability was measured from ~ 1 min of channel activity shortly after obtaining a cell-attached recording ($\sim 1-2$ min). Mean open probability was calculated by integrating the current record and dividing by the number of channels in the patch times the unitary current. For very low activity where simultaneous openings were not observed, we assumed only one channel was in the patch. The abscissa then represents open probability (P) multiplied by the number of channels (N_p). If $N > 1$, P would be overestimated, but any error would be minimal because $P \ll 1\%$. Inset, expansion of the first bin of the probability histogram.

the suction applied to the electrode once again restored activity, although it was less than that recorded at the beginning of the experiment, indicating a slow loss of channel activity occurring over minutes. The results indicate that the activity of all the channels in the patch was reduced by applying suction to the electrode.

Apparently, *mdx* myotubes possess a class of mechano-transducing channels that are open for extended periods of time and close when the membrane is stretched by applying suction to the electrode. To determine whether stretch-inactivation of open mechano-transducing channels is a unique characteristic of *mdx* muscle, we compared the proportions of stretch-activated and stretch-inactivated channels in myotubes from both *mdx* and normal mice. Figure 4a shows a histogram of the fraction of the total number of patches which contained zero or *n* (where *n* is the number of channels per patch) of stretch-activated or stretch-inactivated channels. The number of stretch-activated channels in *mdx* myotubes was roughly half that in normal myotubes. By contrast, 21% of the patches on *mdx* myotubes had stretch-inactivated channel activity, compared with ~2% of the patches (one patch) on normal myotubes. The results show that there is a greater proportion of stretch-inactivated channels in *mdx* myotubes than in normal myotubes.

Can the appearance of channels that are open at rest account for Ca^{2+} leakage into *mdx* myotubes? We addressed this question by comparing the average time a channel is open in patches from normal and *mdx* myotubes regardless of the response of the channel to pressure. Figure 4b shows a frequency histogram of channel open probability obtained from recordings from different cell-attached patches. In 90% of the recordings from normal myotubes, channel open probability without applied pressure was <10%. By contrast, channel openings in myotubes from *mdx* mice divided the channels into two classes: those of low activity (<10% open probability), observed in ~70% of the patches, with a roughly similar distribution as the activity from control myotubes (Fig. 4b, inset), and those of very high activity (~30% of all patches). The recordings from *mdx* myotubes showing high channel activity (for example, Figs 1c and 3) arose from stretch-inactivated channels in those cases where the response to pressure was tested (seven out of nine patches).

Our recordings of single-channel activity from newly formed myotubes from normal and *mdx* mice indicate both possess Ca^{2+} -permeable mechano-transducing ion channels. These channels are likely to account for much of the resting Ca^{2+} entry as they produce the only detectable unitary currents in recordings with divalent cations as the only inward charge carrier in the electrode. The prolonged channel openings in *mdx* myotubes, which often lasted tens of seconds, could allow sufficient Ca^{2+} influx to elevate intracellular Ca^{2+} to pathological levels, because activation of acetylcholine receptors, which are far less permeable to Ca^{2+} (ref. 16), produces a Ca^{2+} -dependent myopathy¹⁷. We found no difference between normal and *mdx* myotubes in the amount of pressure required to rupture the cell membrane (normal myotubes, 177 ± 42 cm Hg; *mdx* myotubes, 189 ± 51 cm Hg; mean $\pm$ s.d., *n* = 36 and 62, respectively), indicating that the membrane of *mdx* myotubes is not mechanically more fragile¹⁸ and that Ca^{2+} entry into myotubes lacking dystrophin might not be due to a general leakiness of the membrane.

Our results show that myotubes lacking dystrophin possess a novel type of mechano-transducing channel that is open for extended periods of time and closes when suction is applied to the electrode. These results provide no information on the origin of the stretch-inactivated channels in *mdx* myotubes. It is interesting to speculate that dystrophin binds to channels⁴ providing a counterforce to the resting membrane tension. In muscle lacking dystrophin, the resting tension in the cell membrane could be sufficient to keep some channels in the open state. Alternatively, stretch-inactivated channels could represent a new population of channels that becomes unmasked by some other

mechanism during the development of differentiated myotubes. Further experiments are required to understand the mechanism linking an absence of dystrophin to the appearance of stretch-inactivated channels. These results, nevertheless, are the first to point to mechano-transducing ion channels as a possible pathophysiological mechanism underlying elevated intracellular Ca^{2+} in dystrophic muscle. □

Received 5 February; accepted 20 February 1990.

- Hoffman, E. P. & Kunkel, L. M. *Neuron* **2**, 1019–1029 (1989).
- Knudson, C. M., Hoffman, E. P., Kahl, S. D., Kunkel, L. & Campbell, K. P. *J. biol. Chem.* **263**, 8480–8484 (1988).
- Watkins, S. C., Hoffman, E. P., Slayter, H. S. & Kunkel, L. M. *Nature* **333**, 863–866 (1988).
- Campbell, K. P. & Kahl, S. D. *Nature* **338**, 259–262 (1989).
- Webster, C., Silberstein, L., Hays, A. P. & Blau, H. M. *Cell* **52**, 503–513 (1988).
- Bonilla, E. *et al. Cell* **54**, 447–452 (1988).
- Bodensteiner, J. B. & Engel, A. G. *Neurology* **28**, 439–446 (1978).
- Fingerman, E., Campisi, J. & Pardee, A. B. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7617–7621 (1984).
- Mongini, T. *et al. Neurology* **38**, 476–480 (1988).
- Bulfield, G., Siller, W. G., Wight, P. A. L. & Moore, K. J. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1189–1192 (1984).
- Turner, P. R., Westwood, T., Regen, C. M., Steinhardt, R. A. *Nature* **335**, 735–738 (1988).
- Brehm, P., Kullberg, R. & Moody-Corbett, F. *J. Physiol.* **350**, 631–648 (1984).
- Guharay, F. & Sachs, F. *J. Physiol.* **352**, 685–701 (1984).
- Franco, A. Jr & Lansman, J. B. *J. Physiol.* (in the press).
- Morris, C. E. & Sigurdson, W. J. *Science* **243**, 807–809 (1989).
- Adams, D. J., Dwyer, T. M. & Hille, B. *J. gen. Physiol.* **75**, 493–510 (1980).
- Leonard, J. P. & Salpeter, M. M. *J. Cell Biol.* **82**, 811–819 (1979).
- Cooper, B. J. & Hamill, O. P. *Soc. Neurosci. (Abstr.)* **15**, 412.5 (1989).
- Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Plügers Arch. ges. Physiol.* **391**, 85–100 (1981).

ACKNOWLEDGEMENTS. We thank T. Elam and P. Slesinger for helpful discussions. This work was supported by the NIH, the Muscular Dystrophy Foundation, and a Basil O'Connor Award from the March of Dimes.

Compartmentalization of cold-stable and acetylated microtubules in the subsynaptic domain of chick skeletal muscle fibre

Bernard J. Jasmin*, Jean-Pierre Changeux† & Jean Cartaud*

* Biologie Cellulaire des Membranes, Institut Jacques Monod, Université Paris VII, Tour 43, 2 Place Jussieu, 75251 Paris Cédex 05, France

† Neurobiologie Moléculaire, Département des Biotechnologies, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cédex 15, France

THE junction between motor nerve and skeletal muscle in vertebrates is characterized by a complex subneural structure^{1,2} in which the nicotinic acetylcholine receptor (AChR) accumulates at a surface density of up to 10,000–20,000 molecules per μm^2 (refs 3–5). Among the mechanisms^{2,6,7} involved in the maintenance of the accumulation of AChR molecules is the localized expression of AChR subunit genes in the sarcoplasmic nuclei⁸ underlying the motor endplate⁹, referred to as 'fundamental' by Ranvier¹⁰. Immunocytochemical analysis has revealed that in innervated muscle fibres, the Golgi apparatus is also localized to subjunctional domains¹¹. We now report an accumulation of cold-stable and acetylated microtubules in the region of the sarcoplasm underlying the endplate. Together, these observations indicate that the subsynaptic sarcoplasm represents a compartment that is specialized both for the transcription, post-translational processing and stabilization of proteins of the postsynaptic membrane, in particular AChR, and for their targeting to the motor endplate.

We used several monoclonal antibodies to detect α -tubulin antigens in cryostat sections of 15-day-old chick anterior latissimus dorsi (ALD) muscle. In muscles fixed in the cold (4 °C), a commercially available monoclonal antibody

(Amersham) and the B-5-1-2 monoclonal antibody¹², both specific for all α -tubulins, stained areas located underneath motor endplates identified by α -bungarotoxin labelling (Figs 1*a* and 2*a*). The immunostaining was associated with microtubules (MT) surrounding the subsynaptic nuclei, extending from the nuclear periphery towards the endplate. Under these experimental conditions, cytoplasmic (extrasynaptic) MT were not consistently detected elsewhere in the muscle fibres. To observe the organization of cytoplasmic MT, which seemed to be cold-unstable, and to further characterize the subsynaptic MT, we incubated ALD muscles at room temperature with taxol to promote MT stability^{13,14}. Subsequent staining with anti- α -tubulin antibodies revealed, in addition to the subsynaptic MT, and in contrast to observations with cold-treated muscles, cytoplasmic labelling parallel to myofibrils (Fig. 1, *b* and *c*). Some

of these MT may have resulted from taxol-induced polymerization of soluble tubulin present in the sarcoplasm. Nonetheless, these data indicate that in chick ALD muscle, cold-labile MT are dispersed throughout the cytoplasm, whereas a subset of cold-stable MT are strictly located underneath motor endplates.

In light of these observations, we used the 6-11B-1 monoclonal antibody specific for acetylated α -tubulin¹⁵. In taxol-treated muscles, this antibody also revealed MT in subsynaptic areas (Fig. 1, *d* and *e*) but, unlike anti- α -tubulin antibodies (Fig. 1, *b* and *c*), it did not label cytoplasmic MT. Again, the labelling of acetylated α -tubulin followed MT arrays extending from the subsynaptic nuclei towards the endplate. Labelling with the 6-11B-1 antibody was similar in subsynaptic areas of cold-treated muscles (results not shown). Therefore, in chick ALD muscle, acetylated MT arrays are compartmentalized in the

FIG. 1 Double-labelling experiments of chick ALD cryostat sections stained for AChR (*a*, *b*, *d*, *e* (left panels)) and MT (*a*, *b*, *c*, *d*, *e* (right panels)): *a*, Synaptic MT identified with B-5-1-2 antibody (specific for all α -tubulins) in cold-treated muscle; *b*, Cytoplasmic and synaptic (arrow) MT identified with B-5-1-2 antibody in taxol-treated muscle; *c*, Cytoplasmic MT stained with B-5-1-2 antibody in taxol-treated muscle. Note the longitudinal organization of the MT; *d* and *e*, Synaptic MT identified with 6-11B-1 antibody (specific for acetylated α -tubulin) in section taken from the same taxol-treated muscle. Note the absence of immunoreactivity with the cytoplasmic MT. Bar, 15 μ m.

METHODS. Fifteen-day-old chick ALD muscles were fixed at 4°C with 3% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), impregnated with 25% (w/v) sucrose and frozen. Sections (4 μ m) were obtained by longitudinally cutting the muscles in a cryostat at -20°C. Double-labelling experiments were conducted using fluorescein isothiocyanate (FITC)-conjugated α -bungarotoxin to label AChR at neuromuscular junctions, and the various monoclonal α -tubulin antibodies revealed with tetramethylrhodamine (TMR) conjugated goat anti-mouse IgG as described in detail elsewhere¹¹. For taxol treatment, muscles were incubated for 60 min at room temperature in MTG buffer¹³ (100 mM MES 2-(*N*-methylthio)-ethanesulphonic acid, pH 6.4, 1 mM EGTA, 1 mM GTP, 0.5 mM MgCl₂) containing 5 μ M taxol (provided by D. Guénard, Institut des Substances Naturelles du CNRS, Gif-sur-Yvette, France). Taxol-treated muscles were fixed at room temperature. Photography was with a Leitz Aristoplan photomicroscope equipped with epifluorescence illumination (filters for FITC and TMR) and with $\times 100$ (N.A. 1.32) immersion optics. Care was taken to ensure that the photographed MT arrays were indeed localized in muscle fibres and not in surrounding tissues.

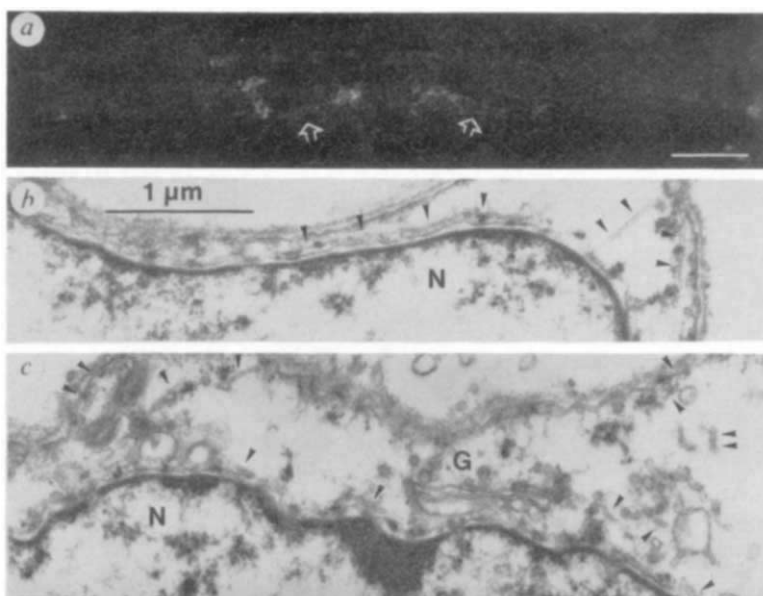
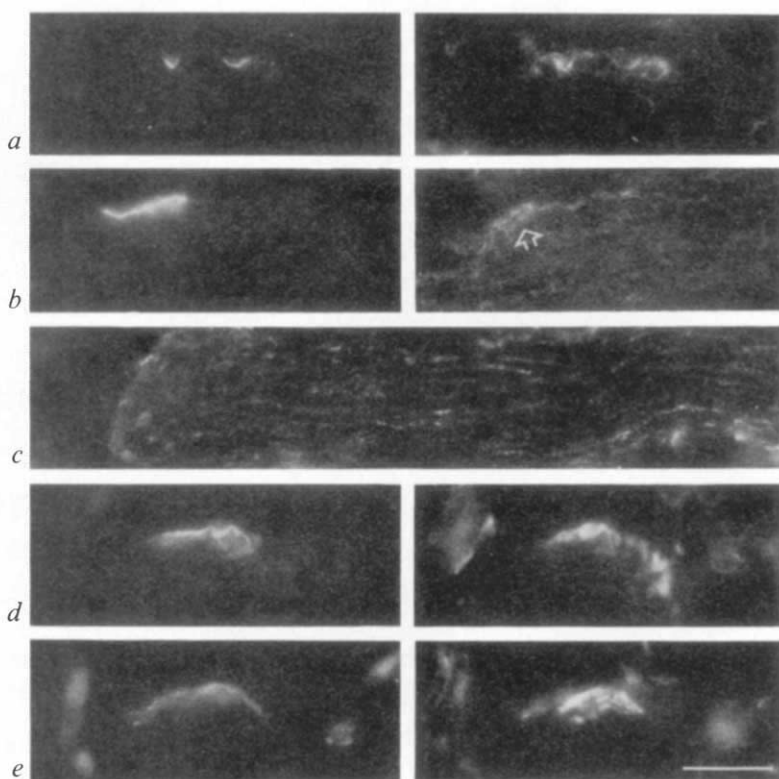


FIG. 2 Synaptic MT organization in chick ALD muscle. *a*, MT arrays identified with B-5-1-2 antibody in cryostat section of cold-treated muscle. Note the perinuclear staining which also extends from the nuclear periphery towards the motor endplate. Arrows indicate nuclei. Bar, 10 μ m. *b* and *c*, Electron micrographs of subsynaptic regions of taxol-treated chick ALD muscle. These images taken at the ultrastructural level demonstrate the occurrence and the subcellular organization of synaptic MT as observed by immunofluorescence. For electron microscopy experiments, muscles were fixed at room temperature with 2% glutaraldehyde, 0.1% tannic acid in 0.1 M cacodylate buffer (pH 7.4), post-fixed with 1% OsO₄, and embedded in epon-araldite. Ultra-thin sections were stained with lead citrate and uranyl acetate, and observed at 80 kV with a Philips 400 electron microscope. Arrows indicate microtubules; N, nuclei; G, Golgi apparatus.

TABLE 1 Distribution and properties of MT in chick skeletal muscle

	Cold-treated		Taxol-treated	
	α -Tubulin	Acetylated α -tubulin	α -Tubulin	Acetylated α -tubulin
Synaptic	+	+	+	+
Cytoplasmic (extrasynaptic)	—	—	+	—

subsynaptic regions of the muscle fibres. A summary of these observations is presented in Table 1.

We confirmed the occurrence and organization of MT in subsynaptic areas (Fig. 2a) at the ultrastructural level. Electron microscopy of chick ALD muscles showed MT both parallel to the nuclear periphery and extending towards the postsynaptic membrane (Fig. 2b). Also, MT arrays were particularly evident close to and around the Golgi apparatus, which is preferentially localized to the motor endplate level in innervated muscle fibre¹¹ (Fig. 2c).

The results presented here indicate that in chick muscle fibres, cold-labile MT that do not contain detectable quantities of acetylated α -tubulin are dispersed in the cytoplasm and, as such, probably represent an unstable population of MT which continuously exchange subunits from the tubulin pool^{16–18}. Conversely, in areas underlying motor endplates, cold-stable and acetylated MT are present. Whether the cold-stable and the acetylated MT represent the same MT population is not known, but some evidence indicates that acetylated MT are more resistant to cold-induced depolymerization^{19,20}. Nonetheless, these characteristics of the subsynaptic MT make them part of stable MT arrays^{12,17,19–22} which appear mostly nongrowing in fibroblasts^{17,18}.

The two subsets of MT in chick skeletal muscle probably have different functions. The properties of the unstable cytoplasmic MT make them suitable for a structural role involving rapid changes in configuration¹⁸ adapted to the various functional demands placed on the muscle fibre. The subsynaptic MT, together with the Golgi apparatus¹¹, are probably involved in the intracellular transport of vesicles and organelles at the endplate level, as generally assumed for stable MT^{16–18,20,23–26}. Such a function is consistent with a model of localized gene expression^{8,9}, protein synthesis, post-translational modifications¹¹ and transport to the endplate (this report) of AChR. The compartmentalization of gene expression in the subsynaptic domain of muscle fibres is supported by evidence from heterokaryons²⁷, and could be at the origin of the focal distribution of fast myosin isoforms observed in slow muscle fibres after ectopic innervation with a fast motor nerve²⁸.

The mechanism whereby a subset of MT is selectively stabilized at the motor endplate level is intriguing. In a recent model^{16,18,22}, it is proposed that without an external morphogenetic signal, MT randomly polymerize within the cell with a high turnover rate; on receiving an external cue at the cell surface, a subset of MT becomes stabilized at the site of signal reception. In skeletal muscle fibres, the early contact of the exploratory motor axons with the myotubes could represent this external cue. Because nerve growth factor induces a selective stabilization and acetylation of MT in PC12 cells^{29,30}, it is plausible that the motor nerve signal produces similar effects. The calcitonin gene-related peptide is a candidate for such a role because it is present in axon terminals of spinal cord motor neurons³¹, and increases the surface level of AChR in chick muscle cells in primary culture^{32,33}. The previously proposed^{2,34} model—that different first messengers regulate distinct intracellular second-messenger pathways in subsynaptic versus extrasynaptic areas of muscle fibres thereby leading to different patterns of gene expression—can therefore be extended to the morphogenesis of stable MT arrays involved in AChR transport. It remains to be seen whether MT stabilization involves differential subsynaptic expression or local distribution of specific micro-

tubule-associated proteins (MAPs), or both of these. The neuromuscular junction is therefore a unique *in vivo* system in which to investigate mechanisms of selective stabilization of MT resulting from cell-cell interactions. □

Received 1 November 1989; accepted 6 February 1990.

1. Couteaux, R. in *The Structure and Function of Muscle* Vol. 2 (ed. Bourne, G. H.) 483–530 (Academic, New York, 1973).
2. Changeux, J.-P., Karsfeld, A. & Heidmann, T. in *The Cellular and Molecular Bases of Learning* (eds Changeux, J.-P. & Konishi, M.) 31–83 (Wiley, London, 1987).
3. Lafer, R., Fontaine, B., Karsfeld, A., Cartaud, J. & Changeux, J.-P. *News Physiol. Sci.* **4**, 5–9 (1989).
4. Salpeter, M. M. & Loring, R. H. *Prog. Neurobiol.* **25**, 297–325 (1985).
5. Changeux, J.-P. et al. in *Molecular Biology of Neuroreceptors and Ion Channels* Vol. H32 (ed. Maelicke, A.) 481–507 (Springer, Berlin, Heidelberg, 1989).
6. Changeux, J.-P. et al. in *Strategy and Prospects in Neuroscience* (ed. Hayaishi, O.) 29–76 (Japan Scientific Societies Press, Tokyo, 1987).
7. Merlie, J. P. & Sanes, J. R. in *Molecular Aspects of Neurobiology* (eds Montalcini, R. L., Calissano, P., Kandel, E. R. & Maggi, A.) 75–80 (Springer, Heidelberg, 1986).
8. Fontaine, B., Sassoon, D., Buckingham, M. & Changeux, J.-P. *EMBO J.* **7**, 603–609 (1988).
9. Merlie, J. P. & Sanes, J. R. *Nature* **317**, 66–68 (1985).
10. Ranvier, L. *Traité Technique d'Histologie* 2nd edn (Savy, Paris, 1888).
11. Jamin, B. J., Cartaud, J., Bornens, M. & Changeux, J.-P. *Proc. natn. Acad. Sci. U.S.A.* **86**, 7218–7222 (1989).
12. Piperno, G., LeDizet, M. & Chang, X. *J. Cell Biol.* **104**, 289–302 (1987).
13. Schiff, P. B., Fant, J. & Horwitz, S. B. *Nature* **277**, 665–667 (1979).
14. Schiff, P. B. & Horwitz, S. B. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1561–1565 (1980).
15. Piperno, G. & Fuller, M. T. *J. Cell Biol.* **101**, 2085–2094 (1985).
16. Kirschner, M. & Mitchison, T. *Cell* **45**, 329–342 (1986).
17. Schulze, E. & Kirschner, M. *J. Cell Biol.* **102**, 1020–1031 (1986).
18. Schulze, E. & Kirschner, M. *J. Cell Biol.* **104**, 277–288 (1987).
19. Greer, K. & Rosenbaum, J. L. in *Cell Movement* Vol. 2 (eds Warner, F. D. & McIntosh, J. R.) 47–66 (Alan R. Liss, New York, 1989).
20. Sale, W. S., Besharse, J. C. & Piperno, G. *Cell Mot. Cytoskel.* **9**, 243–253 (1988).
21. LeDizet, M. & Piperno, G. *J. Cell Biol.* **103**, 13–22 (1986).
22. Schulze, E., Asai, D. J., Bulinski, J. C. & Kirschner, M. *J. Cell Biol.* **105**, 2167–2177 (1987).
23. Linck, R. W. in *Cell Movement* Vol. 2 (eds Warner, F. D. & McIntosh, J. R.) 13–21 (Liss, New York, 1989).
24. Miller, R. H., Lasek, R. J. & Katz, M. J. *Science* **235**, 220–222 (1987).
25. Brady, S. T., Tytell, M. & Lasek, R. J. *J. Cell Biol.* **99**, 1716–1724 (1984).
26. Kirschner, M. W. *Harvey Lect.* **83**, 1–20 (1989).
27. Paviath, G. K., Rich, K., Webster, S. G. & Blau, H. *Nature* **337**, 570–573 (1989).
28. Salviati, G., Biasia, E. & Aloisi, M. *Nature* **322**, 637–639 (1986).
29. Black, M. M. & Greene, L. A. *J. Cell Biol.* **95**, 379–386 (1982).
30. Black, M. M. & Keyser, P. J. *Neurosci.* **7**, 1833–1842 (1987).
31. Matteoli, M. et al. *Proc. natn. Acad. Sci. U.S.A.* **85**, 7366–7370 (1988).
32. Fontaine, B., Karsfeld, A., Hökfelt, T. & Changeux, J.-P. *Neurosci. Lett.* **71**, 59–65 (1986).
33. New, H. V. & Mudge, A. W. *Nature* **323**, 809–811 (1986).
34. Lafer, R. & Changeux, J.-P. *Molec. Neurobiol.* **3**, 1–59 (1989).

ACKNOWLEDGEMENTS. We thank Drs E. L. Benedetti, M. Bornens, R. Couteaux and P. Denoulet for discussions, Dr G. Piperno for providing the 8-5-1-2 and 6-11B-1 antibodies and M. A. Ludosky for expert technical assistance. The work was supported by the CNRS, Université Paris VII, the Collège de France, and the Association Française contre les Myopathies. B.J.J. is a recipient of a postdoctoral fellowship from NATO (NSERC, Canada).

Mitosis-specific phosphorylation causes 83K non-muscle caldesmon to dissociate from microfilaments

Shigeko Yamashiro, Yoshihiko Yamakita, Ryoki Ishikawa & Fumio Matsumura

Department of Molecular Biology and Biochemistry, Rutgers University, Nelson Hall, Piscataway, New Jersey 08854-1059, USA

At mitosis in eukaryotic cells there are profound changes of shape and structure whose causes are almost entirely obscure. What is known is that there are changes in the organization of microfilaments^{1,2}, including the disassembly of microfilament bundles during prophase and the accompanying rounding-up of cultured cells; the formation of transient contractile rings during cytokinesis; and, subsequently, the reassembly of microfilament bundles and the respraying of the two daughter cells. As an initial step towards the biochemical understanding of these events, in which the disassembly and reassembly of microfilaments appear to play an important part, we searched for alterations of the molecular constitution of microfilaments during mitosis. We found that non-muscle caldesmon, a protein with a relative molecular mass (M_r) of 83,000

(83K) which binds to actin and calmodulin, is dissociated from microfilaments during mitosis, apparently as a consequence of phosphorylation. This process may contribute to the changes of shape and structure of cells in mitosis, as caldesmon inhibits actomyosin ATPase.

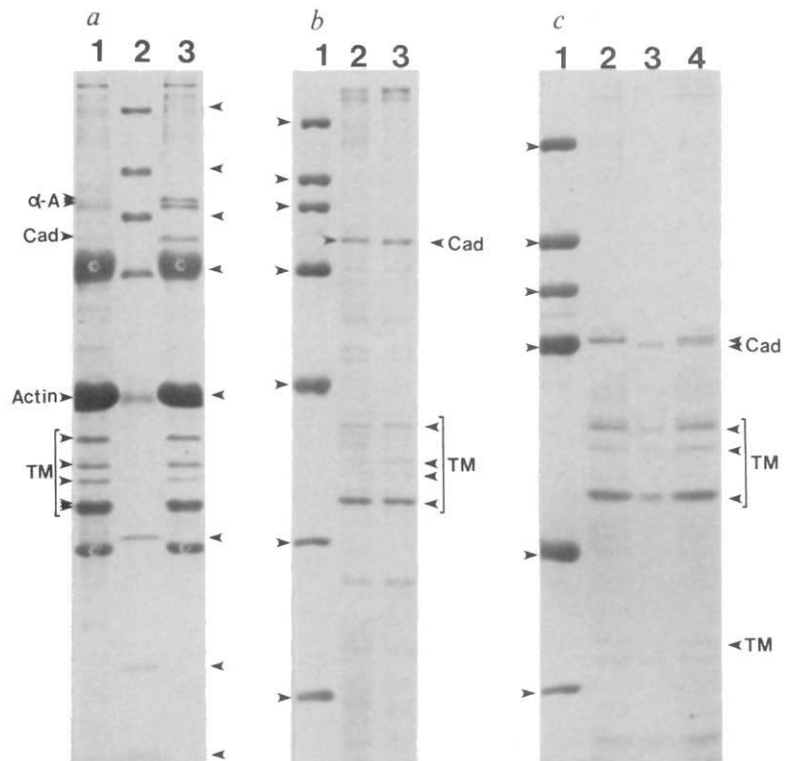
We purified 'intact' microfilaments, from mitotic and non-mitotic cells, by monoclonal antibody-induced aggregation³

FIG. 1 Comparison of levels of microfilament-associated and total caldesmon in mitotic and non-mitotic cells. *a*, SDS-PAGE analysis of microfilament fractions from mitotic (lane 1) and non-mitotic cells (lane 3). *b*, SDS-PAGE analysis of total levels of caldesmon from mitotic (lane 2) and non-mitotic cells (lane 3). *c*, Urea/SDS-PAGE analysis of mitotic (lane 2) and non-mitotic caldesmon (lane 3). Lane 4, simultaneous loading of both mitotic and non-mitotic caldesmons. Lanes 2*a*, 1*b* and 1*c* are *M_r* markers (from top to bottom, 200K, 117K, 94K, 68K, 43K, 30K, 21K, 14K). Asterisks show anti-tropomyosin IgM heavy and light chain. Cad, caldesmon; α -A, α -actinin; TM, tropomyosin.

METHODS. REF-2A cells (simian virus 40-transformed rat embryo-derived fibroblasts¹⁶) were grown to subconfluent levels in Dulbecco's modified Eagle's medium (DMEM) containing 6% newborn calf serum, and treated for 3 h with 0.25 $\mu\text{g ml}^{-1}$ of nocodazole. Mitotic cells were gently detached by moving the media across the dishes with a 10-ml pipette and collected by low-speed centrifugation. Non-mitotic cells were also collected from the dishes after removal of mitotic cells. Microfilaments were prepared by monoclonal antibody-induced aggregation as described previously³. Briefly, cells were extracted with Triton/glycerol solution (0.1 M PIPES, 5 mM MgCl_2 , 0.2 mM EGTA, 0.05% Triton X-100, 4 M glycerol, pH 6.9), and the residual cytoskeleton was homogenized in the presence of 5 mM MgATP. After centrifugation, microfilaments in the supernatants were aggregated into bundles by the incubation with 0.05 volumes of ascite fluids of IV15, anti-tropomyosin monoclonal antibodies; collected by low-speed centrifugation; washed with phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4 , 8 mM Na_2HPO_4 , pH 7.3); and analysed by SDS-PAGE. For a comparison of total levels of caldesmon, mitotic and non-mitotic cells were directly homogenized in PBS containing 5 mM MgATP, 0.2 mM EGTA, 1 mM dithiothreitol (DTT), and 1 mM phenylmethane sulphonyl fluoride. After adjustment to equal protein concentrations, the whole-cell extracts were heated for 10 min at 100°C, cooled on

(Fig. 1*a*). Of the components of the microfilaments of non-mitotic cells, non-muscle caldesmon is apparently absent from the microfilaments of mitotic cells, whereas other components are unaltered. Although there appears to be a decrease in α -actinin in the mitotic microfilaments of this particular preparation, this change is not consistently found.

Is the absence of caldesmon with microfilaments associated



ice for 30 min, and centrifuged for 15 min. The supernatants (heat-stable fractions) were analysed by SDS-PAGE (*b*) or urea/SDS-PAGE (*c*). Urea/SDS-PAGE was carried out as described¹⁷ using 8% polyacrylamide gels containing 6 M urea.

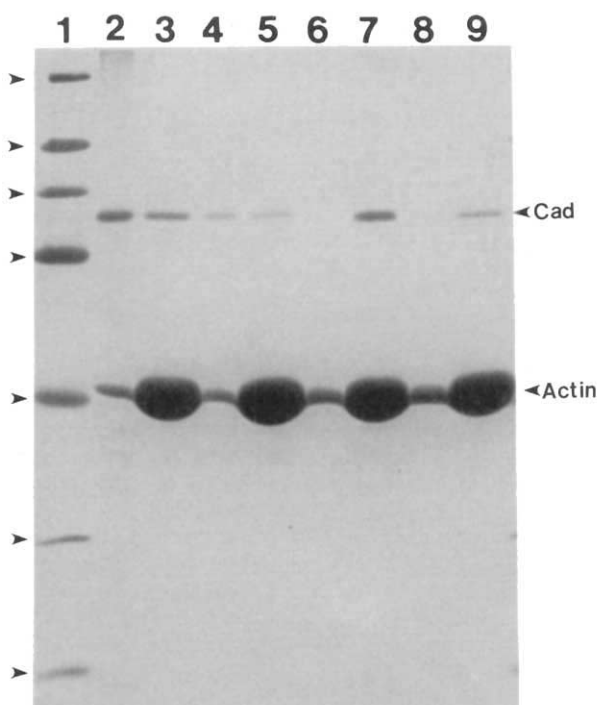


FIG. 2 Actin binding of mitotic and non-mitotic caldesmon. Actin binding was assayed at two different concentrations (lanes 2 and 3, 1 μM mitotic caldesmon; lanes 4 and 5, 0.5 μM mitotic caldesmon; lanes 6 and 7, 1 μM of non-mitotic caldesmon; lanes 8 and 9, 0.5 μM of non-mitotic caldesmon), and supernatants (lanes 2, 4, 6, 8) and pellets (lanes 3, 5, 7, 9) were analysed by SDS-PAGE. Lane 1, *M_r* markers (the same as in Fig. 1).

METHODS. Caldesmons were purified by calmodulin-Sepharose column chromatography¹⁸ from the heat-stable fractions of mitotic and non-mitotic cell extracts prepared as described in the Fig. 1 legend. Caldesmons at final concentrations of either 0.5 μM or 1 μM were incubated with 12 μM of F-actin at room temperature for 1 h in 60 μl of 20 mM imidazole buffer of pH 7.0 containing 100 mM KCl and 0.2 mM DTT. After centrifugation with a Beckman Airfuge (28 p.s.i. $\times$ 20 min), both supernatants and pellets were dissolved in an equivalent volume of SDS sample buffer and analysed by SDS-PAGE.

due to a decrease of total caldesmon? Total amounts of caldesmon can be easily compared by gel analyses of heat-stable fractions of the cell extracts (Fig. 1b, lanes 2 and 3). We found that amounts of caldesmon as well as of tropomyosins are indistinguishable in mitotic and non-mitotic cells, which suggests that caldesmon has substantially less affinity for actin in mitotic than in non-mitotic cells.

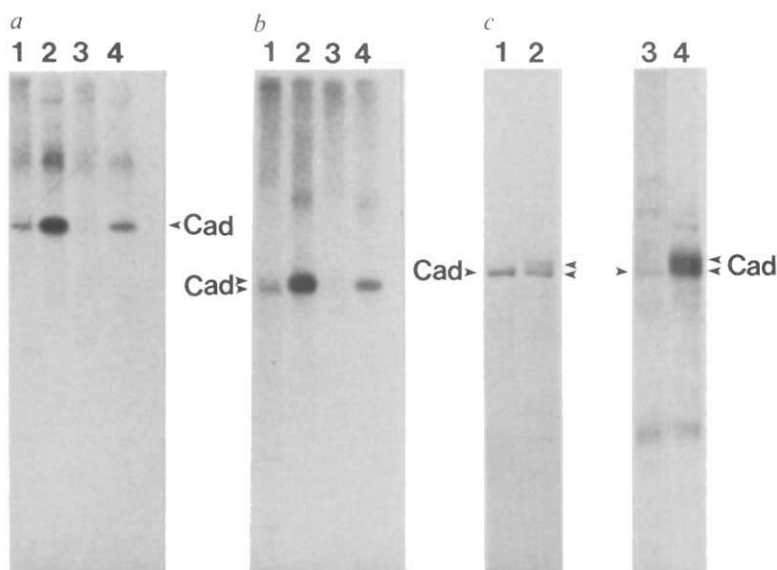
What can explain the dissociation of caldesmon from microfilaments in mitotic cells. One possibility is that the binding affinity of caldesmon is reduced in mitotic cells. Another is that other molecules inhibit actin binding. To distinguish these

possibilities, we examined the actin-binding properties of caldesmons from mitotic and non-mitotic cells at two different concentrations. We found that mitotic caldesmon has much lower affinity for actin than does non-mitotic caldesmon (Fig. 2). Only 35% (0.5 μ M) or 50% (1.0 μ M) of mitotic caldesmon binds to actin, whereas more than 90% of non-mitotic caldesmon binds to actin at either concentration, suggesting that caldesmon may be modified during mitosis.

Further evidence that caldesmon is modified during mitosis was obtained using sodium dodecyl sulphate (SDS) gels containing 6M urea. Although mitotic and non-mitotic caldesmons

FIG. 3 *In vivo* (a, b) and *in vitro* (c) phosphorylation of caldesmon. a, b, Caldesmon recovered by immunoprecipitation from 32 P-labelled mitotic and non-mitotic cell lysates, were analysed by autoradiography of SDS gels (a) and urea/SDS gels (b). Lanes 1, immunoprecipitates from non-mitotic cells with a monoclonal antibody (SM 12) against caldesmon; lanes 2, SM 12 immunoprecipitates from mitotic cells; lanes 3, immunoprecipitates from non-mitotic cells with a polyclonal antibody against caldesmon; lanes 4, immunoprecipitates from mitotic cells with the same antibody. c, *In vitro* phosphorylation of caldesmon by mitotic extracts. Non-mitotic caldesmon was incubated by either mitotic (lanes 2 and 4) or non-mitotic (lanes 1 and 3) cell extracts and analysed by urea/SDS PAGE. Lanes 1 and 2, Coomassie blue staining; lanes 3 and 4, autoradiography.

METHODS. a, b, REF-2A cells (3×100 mm dishes, 5×10^6 cells per dish) were incubated for 3 h in phosphate-free DMEM containing 10% dialysed newborn calf serum, 0.25 μ g ml $^{-1}$ of nocodazole and 0.125 mCi ml $^{-1}$ of 32 P orthophosphoric acid. Mitotic and non-mitotic cells were washed quickly with PBS, lysed in 2 $\times$ SDS sample buffer (100 μ l for mitotic, 900 μ l for non-mitotic cells). These lysates were homogenized by several passages through a 25-gauge needle, heated for 3 min at 100°C, and immediately used for immunoprecipitation using monoclonal and polyclonal antibodies against caldesmon as described previously¹⁸. c, Preparation of mitotic and non-mitotic HeLa cell extracts: HeLa cells were grown to subconfluent levels on eight large culture dishes (245 $\times$ 245 mm, Nunc) in DMEM containing 6% calf serum, and treated with 0.25 μ g ml $^{-1}$ of nocodazole for 16 h. Mitotic and non-mitotic cells were first extracted for 2 min with Triton/glycerol solution (see Fig. 1 legend), and homogenized with an equal volume of PBS containing 0.2 mM EGTA, 5 mM MgCl $_2$, 1 mM phenyl methane sulphonyl fluoride and 1 mM DTT. The supernatants obtained after centrifugation in a



Beckman Airfuge (28 p.s.i. $\times$ 20 min), constituted the mitotic and non-mitotic cell extracts. For *in vitro* phosphorylation, 51 μ g ml $^{-1}$ of non-mitotic caldesmon in 60 μ l of PBS was incubated at room temperature for 1 h with 5 mg ml $^{-1}$ of cell extracts containing 5 mM MgCl $_2$, 0.2 mM EGTA, 2.5 mM ATP, 5 μ Ci [γ - 32 P]ATP (3,000 Ci mmol $^{-1}$, Amersham), and 500 μ M A-kinase inhibitor peptide³ (Sigma). The reaction was stopped by raising the temperature and caldesmon was recovered in the supernatants by low-speed centrifugation.

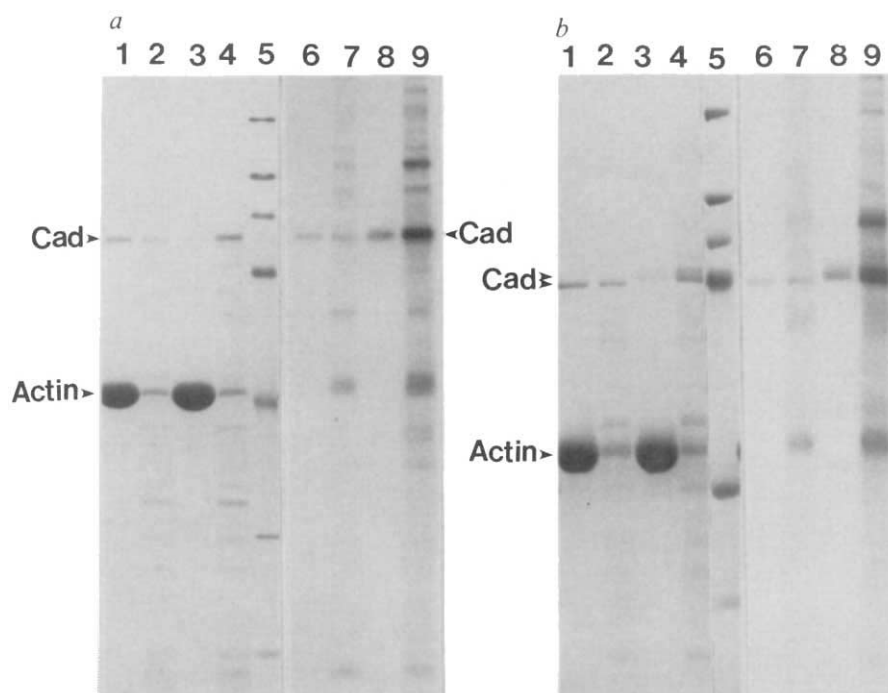


FIG. 4 Reduction of actin-binding affinity of caldesmon by *in vitro* phosphorylation with mitotic cell extracts analysed by SDS-PAGE (a) and urea SDS-PAGE (b). Lanes 1-5, Coomassie blue staining; lanes 6-9, autoradiography; lane 5, M, markers (the same as in Fig. 1); lanes 1, 2, 6 and 7, actin binding of caldesmon treated with non-mitotic cell extracts; lanes 3, 4, 8 and 9, actin binding of caldesmon treated with mitotic extracts.

METHODS. Non-muscle caldesmons were first incubated with either mitotic or non-mitotic cell extracts and recovered as described in the Fig. 3 legend. After addition of 12 μ M of F-actin, the mixtures were incubated at room temperature for 1 h, and then centrifuged with a Beckman Airfuge (28 p.s.i. $\times$ 20 min). Both supernatants (lanes 2, 4, 7 and 9) and pellets (1, 3, 6, and 8) were dissolved in an equivalent volume of SDS sample buffer and run on the gels.

co-migrate on normal SDS gels, mitotic caldesmon is less mobile (Fig. 1c, lane 2) than non-mitotic caldesmon (lane 3) on urea/SDS gels. When both forms of caldesmon were loaded in the same lane, a doublet band appeared (lane 4). The mobility of non-muscle caldesmon also alters on urea/SDS gels: it has an apparent M_r of 83K on SDS gels without urea and an apparent M_r of 67K–68K on urea/SDS gels.

The reduced mobility of mitotic caldesmon suggested that it might be phosphorylated *in vivo*. To investigate this possibility, mitotic and non-mitotic REF52-2A cells were labelled with [32 P]-orthophosphate and caldesmon was prepared by immunoprecipitation from lysates with the same total counts of 32 P radioactivity. Caldesmon in mitotic cells was much more highly phosphorylated (Fig. 3a, lanes 2 and 4) than in non-mitotic cells (Fig. 3a, lanes 1 and 3). Both forms of caldesmon showed the characteristic shift in mobility on urea/SDS gels (Fig. 3b). Mitotic caldesmon is less mobile than non-mitotic caldesmon and both forms move faster on SDS/urea gels than on normal SDS gels. To assess the relative level of caldesmon phosphorylation in mitotic and non-mitotic cells, REF52-2A cells were double-labelled with [32 P] orthophosphate and [3 H]-leucine. We found that caldesmon from mitotic cells contained nine times more 32 P than that from non-mitotic cells (726 c.p.m. for mitotic caldesmon; 78 c.p.m. for non-mitotic caldesmon) although the levels of [3 H] radioactivity were the same (186 c.p.m. for mitotic caldesmon; 171 c.p.m. for non-mitotic caldesmon).

This mitosis-specific phosphorylation of caldesmon can be reconstituted *in vitro* with mitotic cell extracts from HeLa cells. Caldesmon from non-mitotic cells was incubated with mitotic or non-mitotic extracts in the presence of [γ - 32 P]ATP. Caldesmon was recovered in the supernatants by low-speed centrifugation following heat treatment and analysed by SDS/urea polyacrylamide gel electrophoresis (PAGE). Caldesmon was phosphorylated effectively by extracts from mitotic cells (Fig. 3c, lanes 2 and 4) but not by extracts from non-mitotic cells (Fig. 3c, lanes 1 and 3). Furthermore, after phosphorylation by mitotic cell extracts the caldesmon band split into two (lane 2). The slower-moving band was where mitotic caldesmon would be expected to be. Autoradiographic analysis showed that both bands were phosphorylated, suggesting that there is a mitosis-specific kinase activity which causes multiple-step phosphorylation.

Finally, we examined whether this mitosis-specific *in vitro* phosphorylation could reduce the actin-binding affinity of non-mitotic caldesmon. The actin-binding affinity of non-mitotic caldesmon treated with mitotic or non-mitotic HeLa cell extracts was assessed (Fig. 4a, b). We found that caldesmon binds actin much less well after treatment with mitotic cell extracts. The amount of non-muscle caldesmon bound to actin (Fig. 4a, b, lane 3) was reduced to less than one-third of the control level (lane 1). Autoradiographic analysis confirmed that caldesmon was highly phosphorylated by the mitotic extracts (Fig. 4a, b, lanes 8 and 9), but not by the non-mitotic extracts (Fig. 4a, b, lanes 6 and 7).

The mitosis-specific kinase activity does not seem to be protein kinase A, protein kinase C, or the Ca^{2+} /calmodulin-dependent protein kinase for two reasons. Firstly, it was not affected by a variety of inhibitors or activators of these kinases, including cyclic AMP (1 mM), the peptide inhibitor for protein kinase A (0.5 mM)⁴, phosphatidylserine (40 $\mu\text{g ml}^{-1}$), Ca^{2+} (1 mM), EGTA (1 mM), or calmodulin (10 μM) (data not shown). Secondly, although there have been reports of these kinases phosphorylating caldesmon *in vitro*^{5,6}, this does not seem to change the actin-binding affinity of caldesmon. An investigation whether the mitosis-specific caldesmon kinase activity is related to the cdc2 kinase is underway.

What is the physiological function of the dissociation of caldesmon from microfilaments in mitotic cells? Although the *in vivo* functions of caldesmon are not clear it is thought to be involved in the regulation of smooth muscle contraction and

the control of non-muscle cell motility^{7–13}. Ca^{2+} /calmodulin can relieve the inhibition of tropomyosin-stimulated actin-activated myosin ATPase activity by caldesmon as it reduces its actin-binding parity. It is therefore likely that the dissociation of caldesmon from microfilaments after mitosis-specific phosphorylation also releases the inhibition of this ATPase, which may lead to contraction of the actin-myosin system in mitotic cells. Such contraction could have two possible effects on the motility of mitotic cells. Firstly, it may cause rounding-up of cell shape when cells enter the prophase. Secondly, the dissociation of non-muscle caldesmon may be needed later on to modulate the function of contractile rings during cytokinesis.

As well as the regulation of contractility, caldesmon may have some function in the organization of microfilaments in cultured cells. We have shown recently that caldesmon coupled with tropomyosin inhibits both severing and capping activities of gelsolin^{14,15}. The dissociation of caldesmon from microfilaments may release the inhibition of gelsolin activities, resulting in the severing of microfilaments into short filaments. This, in turn, may lead to the disassembly of stress fibres and concomitant morphological alterations during prophase. Further studies are underway to elucidate the biological significance of the mitosis-specific phosphorylation of caldesmon. □

Note added in proof: We find that cdc2 kinase phosphorylates caldesmon.

Received 21 November 1989; accepted 23 January 1990.

- Schroeder, T. E. *Cell Motility* (eds Goldman, R., Pollard, T. & Rosenbaum, J.) 265–278 (Cold Spring Harbor Laboratory, Cold Spring Harbor, (1976).
- Sanger, J. W. & Sanger, J. M. *Cell Motility* (eds Goldman, R., Pollard, T. & Rosenbaum, J.) 1295–1316 (Cold Spring Harbor Laboratory, Cold Spring Harbor, 1976).
- Matsumura, F., Yamashiro-Matsumura, S. & Lin, J. J.-C. *J. biol. Chem.* **258**, 6636–6644 (1983).
- Cheng, H.-C. *et al. J. biol. Chem.* **261**, 989–992 (1986).
- Litchfield, D. W. & Ball, E. H. *J. biol. Chem.* **262**, 8056–8060 (1987).
- Ngai, P. K. & Walsh, M. P. *Biochem. J.* **230**, 695–707 (1985).
- Bretscher, A. *Nature* **321**, 726–727 (1986).
- Horiuchi, K. Y., Miyata, H. & Chacko, S. *Biochem. biophys. Res. Commun.* **136**, 962–968 (1986).
- Ngai, P. K. & Walsh, M. P. *J. biol. Chem.* **259**, 13656–13659 (1984).
- Smith, C. W., Pritchard, K. & Marston, S. B. *J. biol. Chem.* **262**, 116–112 (1987).
- Sobue, K., Takahashi, K. & Wakabayashi, I. *Biochem. biophys. Res. Commun.* **132**, 645–651 (1985).
- Bretscher, A. *J. biol. Chem.* **259**, 12873–12880 (1984).
- Yamashiro-Matsumura, S. & Matsumura, F. *J. Cell Biol.* **106**, 1973–1983 (1988).
- Ishikawa, R., Yamashiro, S. & Matsumura, F. *J. biol. Chem.* **264**, 7490–7497 (1989).
- Ishikawa, R., Yamashiro, S. & Matsumura, F. *J. biol. Chem.* **264**, 16764–16770 (1989).
- McClure, D. B., Hightower, M. J. & Topp, W. C. *Cell. Prolif.* **9**, 345–364 (1982).
- Laemmli, U. K. *Nature* **227**, 680–685 (1970).
- Yamashiro-Matsumura, S., Ishikawa, R. & Matsumura, F. *Protoplasma* (Suppl.) **2**, 9–21 (1988).

ACKNOWLEDGEMENTS. We thank Dr Seth Fidel for critically reading the manuscript. This work is supported by grants from NIH-NCI, Biomedical Research Support, and Johnson & Johnson Discovery Fund. F.M. is a recipient of Research Career Development Award from the NIH.

Activation *in vitro* of NF- κ B by phosphorylation of its inhibitor I κ B

Sankar Ghosh & David Baltimore

Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142, USA, and the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

NUCLEAR factor κ B (NF- κ B), which was first detected by its binding to the κ B site in the immunoglobulin κ -gene enhancer¹, is important for the regulated expression of the κ -gene^{2,3} and is partly responsible for the induction in appropriate cells of interleukin-2 (IL-2)⁴, IL-2 α receptor⁵, β -interferon^{6,7} and serum amyloid A protein⁸. NF- κ B is present as a nuclear DNA-binding protein in B lymphocytes and mature macrophages, but is found in the cytoplasm of many cells in a form unable to bind to DNA⁹. The cytoplasmic form is bound to an inhibitor protein, I κ B, from which it can be released *in vitro* by deoxycholate and other agents¹⁰. Activation of cells by various agents, notably the phorbol esters

that stimulate protein kinase C (PKC), leads to dissociation *in vivo* of the NF- κ B/I κ B complex and migration of NF- κ B to the nucleus⁹. Therefore, it acts as a second messenger system, transducing activation signals from the cytoplasm to the nucleus. To elucidate the mechanism of signal transfer, we have used an *in vitro* system in which addition of purified protein kinases to a partially purified NF- κ B/I κ B complex leads to the activation of the DNA-binding activity of NF- κ B. Using gel retardation assays we found that PKC, cyclic AMP-dependent protein kinase (PKA) and a haem-regulated eIF-2 kinase (HRI) could activate NF- κ B *in vitro*, whereas casein kinase II was ineffective. To determine the target for the protein kinases we purified and characterized both NF- κ B and I κ B and found that I κ B is phosphorylated and inactivated in the presence of PKC and HRI but not PKA.

Because the link between phorbol esters and PKC activation is well established¹¹, we tested the effect of PKC on the NF- κ B/I κ B complex. Initially we added purified protein kinases to crude cytosol from the mouse pre-B cell line 70Z/3, which contains readily detectable amounts of deoxycholate-activatable NF- κ B, sequestered as an NF- κ B/I κ B complex⁹. In contrast to a published report¹², however, large amounts of both PKC and PKA did not activate NF- κ B (data not shown). Because crude cytosolic extracts contain many other substrates for the protein kinases and could also contain inhibitors such as phosphatases, we partially purified the NF- κ B/I κ B complex from cytosolic extracts of 70Z/3 cells. Purification was ~200-fold, because further chromatography led to increasing spontaneous dissociation of the complex.

When increasing amounts of PKC, purified from rat brains as described previously^{13,14}, was added to the partially purified NF- κ B/I κ B complex, significant activation of NF- κ B was observed in an electrophoretic mobility shift assay (EMSA; Fig. 1, lanes 1–5). Almost 75% of the deoxycholate-activatable NF- κ B was activated by 1 unit of PKC (compare lanes 5 and 15 of Fig. 1). The DNA-protein complex was competed away by a specific oligonucleotide but not by an oligonucleotide in which two of the crucial G residues in the κ B site were mutated (Fig. 1, lanes 8–14). The activation was dependent on ATP (Fig. 1, lane 6) and was significantly inhibited by the inclusion of 50 μ M protein kinase inhibitor H-7 (Fig. 1, lane 7)¹⁵. Partially purified α - and γ -subtypes of PKC (produced by expression of complementary DNAs in COS cells and a gift from Dr John Knopf, Genetics Institute, Massachusetts)¹⁶ could also activate NF- κ B in the system we used (data not shown).

To determine if the effect was specific for PKC or could be produced by other serine-threonine protein kinases, we tested PKA, casein kinase II (CK-II) and HRI in the activation reaction. PKA was effective in activating NF- κ B, although the amount of kinase activity (as measured by phosphorylation of exogenous histone substrate) needed was significantly higher than for PKC (Fig. 2, lanes 1 to 5). Activation was dependent on ATP (lane 6) and could be blocked by preincubating the kinase with a peptide substrate inhibitor of PKA (lane 7). The reaction with PKA proceeded slowly with maximum activation occurring after about 10 mins, whereas the reaction with PKC occurred very rapidly (data not shown). HRI, a haem-regulated kinase that phosphorylates the translation initiation factor eIF-2 similarly to double-stranded RNA-activated kinase¹⁷, also activated NF- κ B (Fig. 2, lanes 10 and 11). CK-II, an abundant cytosolic kinase¹⁸, was however totally ineffective in activating NF- κ B, demonstrating that not all protein kinases can activate NF- κ B (Fig. 2, lanes 8 and 9). Kinase reactions performed with [γ -³²P]ATP indicated that all the kinases, including CK-II, phosphorylated several polypeptides in the partially purified NF- κ B/I κ B preparation (data not shown).

The NF- κ B/I κ B complex used in these experiments contained many polypeptides when visualized by silver-staining of SDS-polyacrylamide gels or by labelling with [γ -³²P]ATP and kinases (data not shown). This heterogeneity, coupled with the extremely low abundance of both NF- κ B and I κ B, prevented us

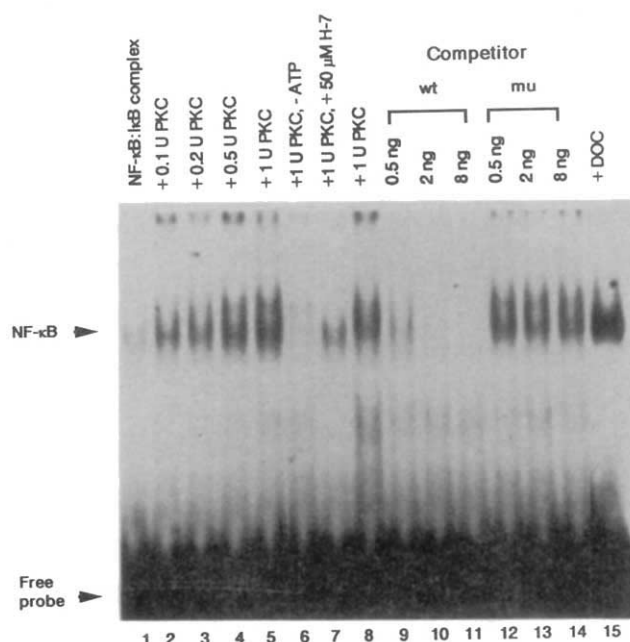


FIG. 1 Activation of NF- κ B by PKC. The glycerol gradient fraction of NF- κ B/I κ B complex was used for kinase reactions. NF- κ B released from the complex was assayed by an electrophoretic mobility shift assay (EMSA) using a radiolabelled oligonucleotide containing the κ B site from the immunoglobulin κ -gene enhancer. Untreated complex (lane 1), with increasing amounts of PKC added to the complex (lanes 2–5). Reactions were also performed in the absence of ATP (lane 6), in the presence of 50 μ M H-7 (lane 7), with increasing amounts of unlabelled oligonucleotide containing the wild-type (wt, lanes 9–11) or the mutant (mu, lanes 12–14) κ B site added to the DNA-binding mixture. The position of labelled DNA bound to NF- κ B was determined by treating the complex with DOC before assaying (lane 15).

METHODS. Kinase reactions were performed in a total volume of 10 μ l buffer containing 100 mM KCl, 30 mM Tris-HCl (pH 7.5), 6 mM MgCl₂, 0.5 mM CaCl₂, 10 μ g ml⁻¹ phosphatidyl serine and 1 μ g ml⁻¹ diolefin (freshly sonicated), 200 μ M ATP and the NF- κ B/I κ B complex. Reactions were initiated by adding the kinase fraction and the reaction mixtures were incubated at 30 °C for 20 min, after which 10 μ l DNA-binding mix¹ containing the labelled probe was added. After the binding reactions, the samples were analysed by EMSA. Fluorograms of the dried gels are shown. Partial purification of NF- κ B/I κ B complex from 70Z/3 cells: All operations were performed at 4 °C and, unless indicated all buffers contained the following protease inhibitors: 0.5 mM PMSF, 1 μ g ml⁻¹ leupeptin, 1 μ g ml⁻¹ pepstatin, 0.01 U ml⁻¹ aprotinin and 1 μ g ml⁻¹ antipain. Cytosol prepared from 3 $\times$ 10¹⁰ 70Z/3 cells¹⁰, was subjected to 0–40% ammonium sulphate fractionation. The precipitate was dissolved in a minimal volume of buffer A (20 mM Tris-HCl (pH 7.5), 1 mM DTT, 0.5 mM EDTA and 10% glycerol) with 100 mM KCl, and dialysed overnight against a large excess of the same buffer. It was then mixed with 100 ml DEAE Sephacel previously equilibrated with buffer A and 280 mM KCl and packed in a column. The flow-through and wash were collected and after dialysis loaded onto a 100 ml DEAE-Sephacel column previously equilibrated with buffer A and 90 mM KCl. The bound proteins were eluted with a salt gradient from 90 mM to 350 mM KCl. NF- κ B/I κ B was assayed by adding 0.8% DOC to the fractions, followed by 1.2% Nonidet P-40 (NP-40) and the κ B DNA probe. After incubating at room temperature for 20 min, reactions were analysed for DNA binding by EMSA⁹. NF- κ B/I κ B eluted as a broad peak at about 140 mM KCl. The peak of activity was pooled, dialysed and loaded onto a 30 ml phosphocellulose column equilibrated with buffer B (20 mM potassium phosphate, pH 7.2, 1 mM DTT, 0.5 mM EDTA and 10% glycerol) and 100 mM KCl. The bulk of the activity was in the flow-through and was loaded onto a 20 ml heparin-sepharose column equilibrated with buffer B and 100 mM KCl. The main portion of the activity was again in the flow-through, which was diluted to twice the volume with buffer A (containing no KCl) and concentrated by binding and eluting from a 4-ml DEAE-Sephacel column in a total of 3 ml. This was further concentrated to ~1 ml using Centricon concentrators. A 200- μ l portion of the concentrated pool was loaded onto 5 ml, 10–30% glycerol gradients in buffer A and 100 mM KCl. The gradients were centrifuged for 36 h at 2 °C in a SW50.1 rotor at 45,000 r.p.m. Fractions of 200 μ l were collected and the total NF- κ B activity eluted in a total of six fractions in each gradient.

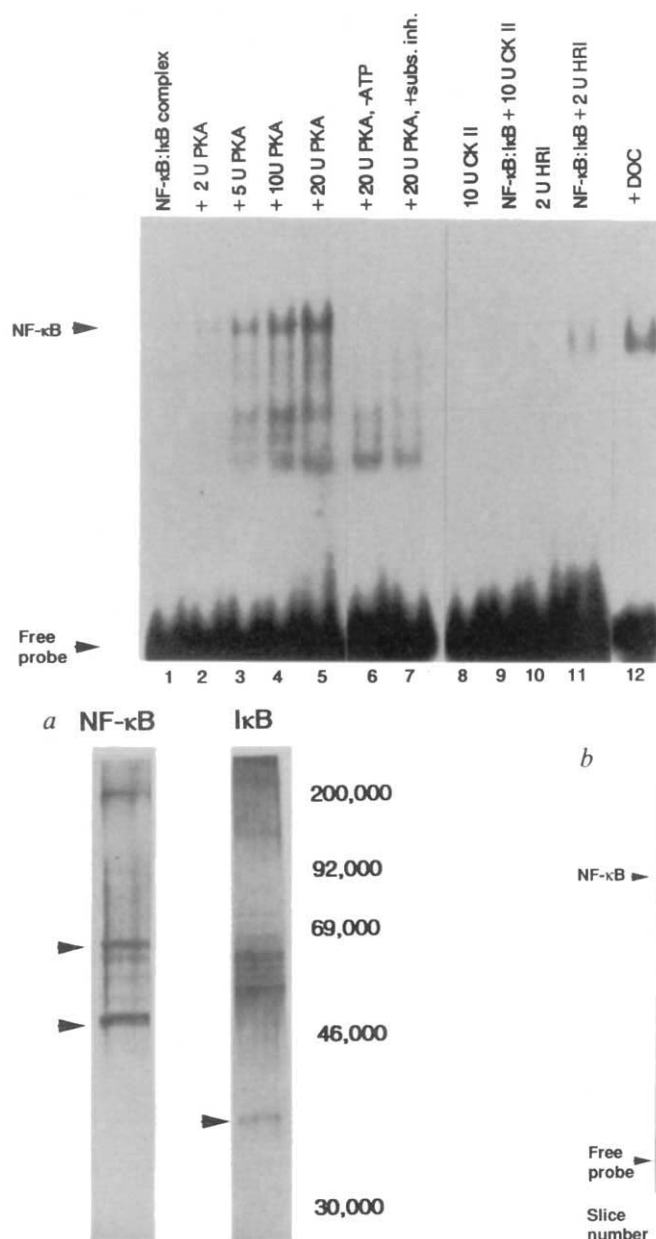


FIG. 3 Molecular size of I κ B. *a*, SDS-PAGE of NF- κ B and I κ B: 500 μ l of an affinity-purified NF- κ B and 500 μ l mono-Q I κ B fraction were precipitated by acetone, and after dissolving in SDS sample buffer, were analysed on an 8.75% SDS-polyacrylamide gel. Proteins on the gel were stained with silver. The 50K and 65K subunits of NF- κ B and the 35K band for I κ B are indicated by arrowheads. The bands between the 69K and 46K markers are probably keratins or other contaminating proteins, because they were present in all fractions. They are less visible in the NF- κ B lane because the higher amount of NF- κ B protein analysed allowed us to avoid saturation-staining with silver. *b*, Renaturation of I κ B activity after SDS-PAGE. The mono-Q fraction (1 ml) was precipitated with acetone and subjected to SDS-PAGE on an 8.75% mini gel. The gel had been pre-run briefly with 0.1 mM sodium thioglycolate in the upper running buffer. After electrophoresis, the gel was cut into 16 slices of $\sim$ 1 mm between the M_r markers of 92K to 21.5K. Proteins were eluted, denatured and renatured essentially as described before^{10,21,22} and assayed for I κ B activity. A fluorogram of the EMSA gel is shown. The migration positions of the prestained M_r markers are indicated.

METHODS. Purification of I κ B from rabbit lung cytosol. 250 g rabbit lungs (Pel Freez) were partially thawed and homogenized in two portions with 500 ml buffer containing 50 mM KCl, 20 mM Tris-HCl (pH 7.5), 1 mM DTT, 0.5 mM EDTA and 0.25 M sucrose. The homogenate was centrifuged at 10,000g for 30 min and the supernatant collected by filtering through four layers of cheese cloth and centrifuged at 45,000 r.p.m. in 50.2Ti rotors for 3 h. After centrifugation, the upper fatty layer was discarded and the supernatant precipitated in 0–40% ammonium sulphate and passed through two DEAE-Sephacel columns (375 ml each). The DEAE gradient pool was

FIG. 2 Effect of other protein kinases on activation of NF- κ B. The partially purified NF- κ B/I κ B complex was treated with no kinase (lane 1) or with increasing amounts of PKA (lanes 2–5). Reactions were also performed with PKA but without ATP (lane 6), or with PKA, ATP and 20 U of a peptide substrate inhibitor (subs. inh.; lane 7). Lane 8, 10 U CK-II alone; lane 9, CK-II with the complex. Lane 10, 2 U HRI alone, whereas lane 11, kinase with the complex (the lower bands in the PKA-containing lanes are due to a nonspecific DNA-binding protein present variably in the commercial PKA preparations). Lane 12, complex treated with DOC to reveal NF- κ B. PKA catalytic subunit, purified from bovine heart, was purchased from Sigma. HRI was purified from rabbit reticulocyte ribosomal salt wash²⁴ (gift from Dr U. Maitra, Albert Einstein College of Medicine, New York), whereas CK-II was purified from rabbit reticulocyte supernatant¹⁸. The NF- κ B which is activated by the kinases migrates slightly slower than the DOC-activated complex.

METHODS. Kinase reactions were performed in a total volume of 10 μ l in buffer containing 75 mM KCl, 30 mM Tris-HCl, pH 7.5, 6 mM MgCl₂, 200 μ M ATP and the NF- κ B/I κ B complex. After adding the kinase, fraction reactions were incubated at 30 °C for 20 min. Binding to the DNA probe was then allowed for 20 min at room temperature followed by EMSA. Fluorograms of the dried gels are shown.

dialysed and loaded onto a 100 ml CM-Sephadex column equilibrated with buffer B and 100 mM KCl. The flow-through and wash were collected and concentrated to 10 ml by 0–45% ammonium sulphate precipitation. All buffers from this stage onward contained 0.1% NP-40. The concentrated material was fractionated in two portions on a 250 ml Sephacryl S-200HR gel filtration column. The peak fractions of NF- κ B/I κ B were pooled and DOC added to a final concentration of 0.8% before mixing with 120 ml Q-Sepharose equilibrated with buffer A and 100 mM KCl. The slurry was mixed for 5 min after which NP-40 was added to a final concentration of 1.2%, mixed for an additional 10 min, before packing in a column. The column was then washed extensively with the equilibrating buffer and the bound proteins eluted with a salt gradient from 100 to 500 mM KCl. NF- κ B was assayed by its binding to a labelled DNA probe containing the κ B site in an EMSA⁹. I κ B was assayed by its ability to block the binding of NF- κ B to its DNA-binding site, when preincubated with NF- κ B and analysed by EMSA²³. NF- κ B was primarily in the flow-through and early region of the gradient whereas I κ B eluted later at a salt concentration of 320 mM. The active I κ B fractions were pooled, adjusted to 0.25 M (NH₄)₂SO₄ and loaded onto a 10 ml phenyl sepharose column. Proteins were eluted by a gradient from 0.25 M (NH₄)₂SO₄ to 50% ethylene glycol. I κ B-containing fractions were pooled and loaded onto a 5-ml hydroxylapatite column equilibrated with buffer B. Bound proteins were eluted with a gradient from buffer B to buffer B with 500 mM potassium phosphate. Active fractions were pooled, diluted with buffer A and loaded onto a 1-ml mono-Q column which was developed with a salt gradient from 50 mM to 400 mM KCl. The peak fractions of I κ B activity were used for further analysis as described.

from observing the phosphorylation of the relevant polypeptides by $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. Because we could not purify the NF- κB /I κB complex beyond this stage, we instead purified both NF- κB and I κB individually to near homogeneity for use in the kinase reactions. NF- κB has been purified from nuclear extracts^{19,20} and cytosol²¹, but I κB has not been molecularly characterized. To purify both components from cytosolic extracts, we partially purified the NF- κB /I κB complex as described above, dissociated the complex with deoxycholate, separated the components on a Q-Sepharose ion exchange column and purified each of them individually. Rabbit lung was used as a tissue source because it was available in large amounts and contained sig-

nificant amounts of NF- κB /I κB . The dissociated NF- κB was purified by sequential oligonucleotide affinity chromatography²¹, to yield a highly purified preparation which contained subunits of relative molecular mass 65,000 (M_r 65K) and 50K (Fig. 3a). The Q-Sepharose I κB fraction, free of NF- κB , was purified to near homogeneity by fractionation on phenyl Sepharose, hydroxylapatite and mono-Q ion exchange chromatography. We obtained ~50 ng purified I κB from 250 g tissue. Analysis of the most purified preparation on a silver-stained SDS-polyacrylamide gel, revealed a band of ~35K and a few other bands (Fig. 3a). To determine whether this stained band was I κB , we fractionated the polypeptides by SDS-PAGE,

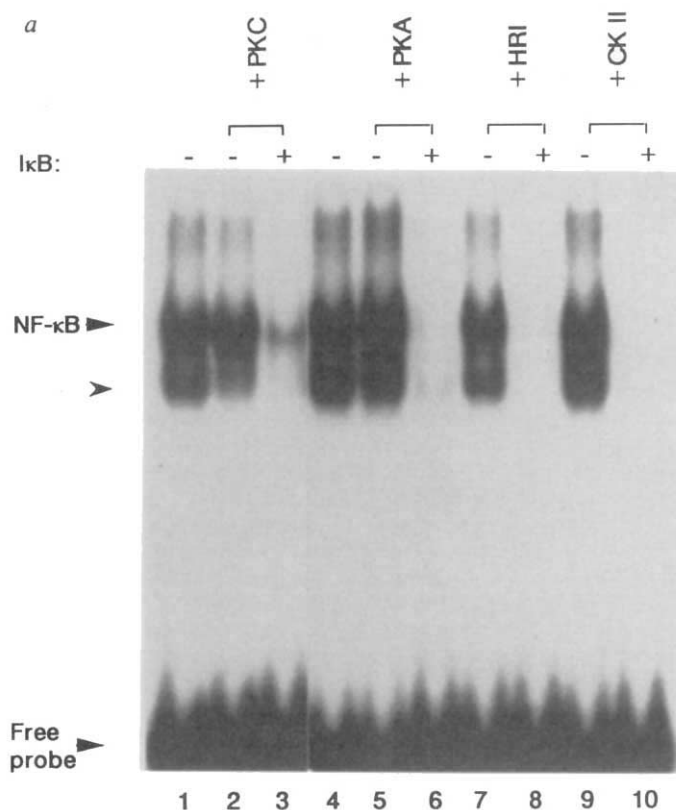
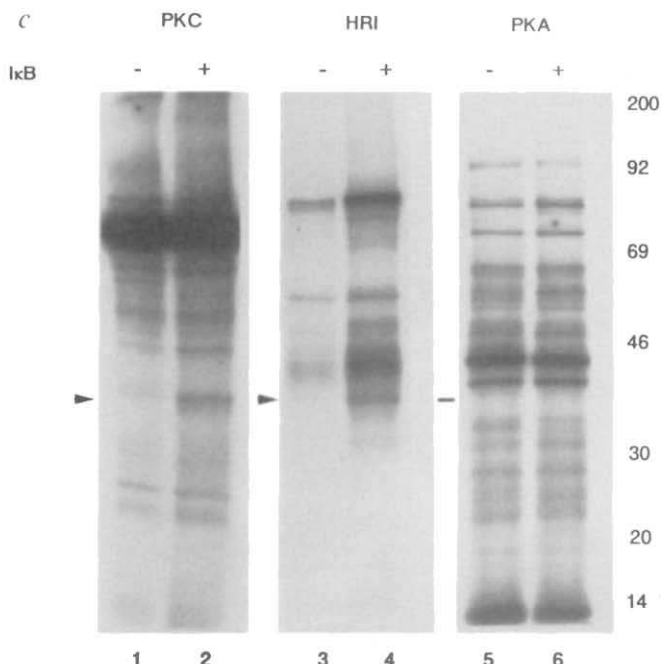
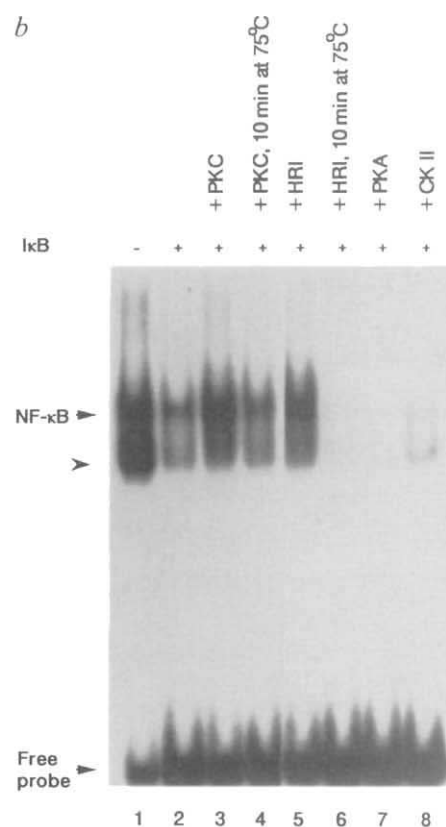


FIG. 4 I κB is the target for protein kinases. *a*, Effect of protein kinases on NF- κB . Affinity-purified NF- κB was treated with kinases before the addition of I κB in lanes 3, 6, 8 and 10. Lanes 1–3 were performed in reaction buffers for PKC (with lipids and calcium). Lanes 1 and 4, NF- κB alone; lanes 2, 5, 7 and 9, NF- κB with PKC (lane 2), PKA (lane 5), HRI (lane 7) and CK-II (lane 9). Also, NF- κB was treated with PKC (lane 3), PKA (lane 6), HRI (lane 8), CK-II (lane 10), followed by I κB . After incubation with kinases, the reaction mixtures were chilled and adjusted to 0.2% DOC/0.2% NP-40. (The lower retarded band is due to the 50K DNA-binding subunit of NF- κB without the 65K subunit²¹). *b*, Effect of protein kinases on I κB . Mono Q I κB (2 μl) was treated with kinases in 10- μl reaction mixtures under standard conditions. Lane 1, NF- κB alone. Lanes 2–8, NF- κB with untreated I κB (lane 2), I κB treated with PKC (lane 3), I κB treated with PKC previously heated at 75 °C for 10 min (lane 4), I κB treated with HRI (lane 5), I κB treated with HRI previously heated at 75 °C for 10 min (lane 6), I κB treated with PKA (lane 7) and I κB treated with CK II (lane 8). The difference in inhibition between the PKC lanes and the others is due to the lipids in the PKC reaction buffers which partially inhibit I κB activity. The amount of I κB used was limiting and was titrated to a level just sufficient to completely inhibit the NF- κB used in the reactions. *c*, Phosphorylation of I κB with protein kinases and $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. Mono Q I κB (50 μl) was phosphorylated by the various kinases in standard reaction conditions with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (7,000 Ci mmol⁻¹). The samples were then precipitated with acetone, dissolved in SDS sample buffer and analysed on an 8.75% polyacrylamide gel. After running, the gel was dried and exposed for autoradiography.



cut the gel into 1-mm pieces and eluted the proteins from the gel slices. The eluted proteins were denatured by guanidinium hydrochloride, renatured by dilution into binding buffer and assayed for I κ B activity²². Activity was detected only in the region of 35K in two slices, with a small amount in the slice just ahead (Fig. 3b). Therefore I κ B activity from rabbit tissue is associated with a 35K protein. Further analysis of purified I κ B by centrifugation on a 5–20% glycerol gradient indicated an M_r of ~35K for I κ B activity. Gel filtration on an FPLC Superose-12 column, however, indicated a size of ~70K. This larger size of I κ B is consistent with the reported value from G-200 gel filtration²³ and might be due to dimerization. The purified protein also displayed the same specificity properties as the I κ B activity in crude cytosolic fractions²³.

Availability of both pure NF- κ B and I κ B allowed us to study the effect of the various kinases on the activity of these proteins. The ability of I κ B to inhibit the DNA-binding activity of NF- κ B was unaffected by treatment of pure NF- κ B with the protein kinases under standard conditions (Fig. 4a). By contrast, treatment with PKC and HRI inhibited the ability of I κ B to block the DNA-binding activity of pure NF- κ B (Fig. 4b, lanes 3 and 5). As a control, the kinases were heated at 75 °C for 10 min before adding to the reaction mixture and such heat-treated kinases could not inactivate I κ B (Fig. 4b, lanes 4 and 6). As expected, CK-II could not inactivate I κ B (Fig. 4b, lane 8), but surprisingly PKA was also totally ineffective on the purified I κ B protein (Fig. 4b, lane 7). This result indicates that the purified preparation of I κ B lacks a component necessary for PKA to act.

To determine whether PKC and HRI were actually phosphorylating the I κ B protein, the kinase reactions were performed with [γ -³²P]ATP. Both PKC and HRI phosphorylated a polypeptide of 35K that corresponded to the exact region from which I κ B activity could be eluted (Fig. 4c; the observed background in the kinase-only lanes is due to trace contaminants in the kinase preparations which are efficiently phosphorylated by the kinases). In addition, PKA did not phosphorylate any protein of about 35K, thereby strengthening the evidence that pure I κ B cannot be phosphorylated and inactivated by PKA. Therefore, PKC and HRI can directly phosphorylate I κ B and our results show that the phosphorylated I κ B cannot inhibit the DNA-binding activity of NF- κ B.

As suggested earlier²³ and in our study, phosphorylation of I κ B causes a change which prevents it from associating with NF- κ B. By extension, we believe that phosphorylation of I κ B in an NF- κ B/I κ B protein complex can free NF- κ B from inhibition, allowing it to translocate to the nucleus and activate its target genes. Among the different kinases, PKC and HRI seem to act directly on I κ B, whereas PKA either works indirectly through an intermediate component or it acts only on the NF- κ B/I κ B complex and not free I κ B. This correlates well with the known activation of NF- κ B by phorbol esters (activators of PKC), double-stranded RNA (which activates a kinase closely related in specificity to HRI) and IL-1, which is thought to activate PKA²⁵. Although several mechanisms exist for converting inactive transcription factors into active ones, the NF- κ B model, whereby phosphorylation of an inhibitor blocks its interaction with the factor, is so far unique. □

15. Kawamoto, S. & Hidaka, H. *Biochem. biophys. Res. Commun.* **125**, 258–264 (1984).
16. Knopf, J. L. *et al. Cell* **46**, 491–502 (1986).
17. Moldave, K. A. *Rev. Biochem.* **54**, 1109–1149 (1985).
18. Hathaway, G. M., Lundak, T. S., Tahara, S. M. & Traugh, J. A. *Meth. Enzym.* **60**, 495–511 (1979).
19. Lenardo, M. J., Kuang, A., Gifford, A. & Baltimore, D. *Proc. natn. Acad. Sci. U.S.A.* **85**, 8825–8829 (1988).
20. Kawakami, K., Scheidereit, C. & Roeder, R. G. *Proc. natn. Acad. Sci. U.S.A.* **85**, 4700–4704 (1988).
21. Baeuerle, P. A. & Baltimore, D. *Genes Dev.* **3**, 1689–1698 (1989).
22. Hager, D. A. & Burgess, R. R. *Analyt. Biochem.* **109**, 76–78 (1980).
23. Baeuerle, P. A. & Baltimore, D. *Science* **242**, 540–545 (1988).
24. Trachsel, H., Ranu, R. S. & London, I. M. *Meth. Enzym.* **60**, 485–495 (1979).
25. Shirakawa, F. *et al. Molec. cell. Biol.* **9**, 959–964 (1989).

ACKNOWLEDGEMENTS. We thank Drs S. Smale, A. DeFranco, M. Schissel, A. Winoto, C. Murre and X. H. Sun for critical reading of the manuscript. The work was supported by a postdoctoral fellowship to S.G. from the Irving Institute for Medical Research, and the NIH (D.B.).

Transcriptional silencing and thermoregulation of gene expression in *Escherichia coli*

Mikael Göransson, Berit Söndén, Peter Nilsson, Björn Dagberg, Kristina Forsman, Karin Emanuelsson & Bernt Eric Uhlin*

Department of Microbiology, University of Umeå, S-901 87 Umeå, Sweden

EXPRESSION of specific adhesive properties by bacteria in general seems to be regulated to fit the environmental conditions. An example is the transcriptional regulation of digalactoside-specific binding by uropathogenic strains of *Escherichia coli*¹. The fimbrial structures (pili) on the bacterial surface carry the adhesin and are present during growth at 37 °C but are not produced by cells at lower temperatures, such as 25 °C. Thermoregulation of expression is due to temperature-dependent transcription of a regulatory cistron in the pilus-adhesin gene cluster. We have now identified and characterized a new regulatory locus (*drdX*) and show that a histone-like bacterial protein has an important role in this novel example of thermoregulation of transcription.

The transcriptional regulation of a cloned pilus-adhesin determinant (the *pap* genes) from uropathogenic *E. coli* was monitored by the use of *lacZ* operon fusions and by analysis of messenger RNA in northern blot experiments^{1–3}. The polycistronic *papB* operon (including the major pilus subunit gene, *papA*) and the monocistronic *papI* operon are divergently oriented, and transcription of both was turned off at 26 °C, in comparison to 37 °C. Our previous studies suggested that the level of mRNA encoding the PapI activator is limiting at low growth temperatures and that thermoregulation of pilus adhesin expression is, at least partly, due to the transcriptional control of the *papI* operon¹. Using *pap-lacZ* operon fusion constructs in *E. coli* K-12 host strains, we searched for mutants which might have altered regulation of *pap* transcription. A spontaneously occurring class of mutants showing derepressed expression of a *pap-lacZ* construct at 37 °C was found after growth of strain MC1029 (ref. 4) carrying the operon fusion. The derepressing mutation was mapped to the vicinity of the *trp* region of the *E. coli* chromosome (our unpublished experiments; also see below). An isolate denoted HMG5 was used for further characterization and the mutant locus was denoted *drdX* (for derepressed expression). In addition to the derepression of *pap* expression, the *drdX* mutation altered the growth properties of the bacteria such that they grow slower than the wild type at 30 °C or below, irrespective of whether they carried the *pap* plasmids or not.

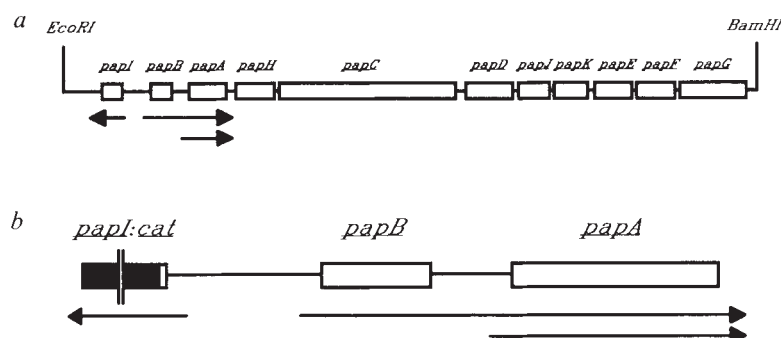
A *papI-cat* operon fusion (Fig. 1) was used to study how the *drdX* mutation affects *papI* transcription at different temperatures. As shown in Table 1, the *papI* transcription was clearly derepressed in the mutant strain. The level of expression

Received 15 January; accepted 26 February 1990.

1. Sen, R. & Baltimore, D. *Cell* **46**, 705–716 (1986).
2. Lenardo, M. J., Pierce, J. W. & Baltimore, D. *Science* **236**, 1573–1577 (1987).
3. Atchison, M. & Perry, R. P. *Cell* **48**, 121–128 (1987).
4. Hoyos, B. *et al. Science* **244**, 457–460 (1989).
5. Bohlman, E. *et al. Cell* **53**, 827–836 (1988).
6. Visvanathan, K. V. & Goodbourn, S. *EMBO J.* **8**, 1129–1138 (1989).
7. Lenardo, M. J., Fan, C. M., Maniatis, T. & Baltimore, D. *Cell* **57**, 287–294 (1989).
8. Edbrooke, M. R., Burt, D. W., Cheshire, J. K. & Woo, P. *Molec. cell. Biol.* **9**, 1908–1916 (1989).
9. Sen, R. & Baltimore, D. *Cell* **47**, 921–928 (1986).
10. Baeuerle, P. A. & Baltimore, D. *Cell* **53**, 211–217 (1988).
11. Kikkawa, Y. & Nishizuka, Y. A. *Rev. Cell Biol.* **2**, 149–178 (1986).
12. Shirakawa, F. & Mizel, S. B. *Molec. cell. Biol.* **9**, 2424–2430 (1989).
13. Woodgett, J. R. & Hunter, T. *J. biol. Chem.* **262**, 4836–4843 (1987).
14. Huang, K.-P., Nakabayashi, H. & Huang, F. L. *Proc. natn. Acad. Sci. U.S.A.* **83**, 8535–8539 (1986).

* To whom correspondence should be addressed.

FIG. 1 Genetic organization of the Pap pilus-adhesin determinant and effect of a *drdX* mutation on expression of a *papI-cat* operon fusion. *a*, Map of genes *papA-K*. *b*, Transcriptional organization (horizontal arrows) in the *papI-B-A* region^{3,27}. The *papI-cat* operon fusion (plasmid pBEU310) was constructed using the vector plasmid pKK232-8 (Pharmacia) (unpublished experiments).



in the *drdX* mutant was higher at both 37 °C and 26 °C than the level of a wild-type strain at 37 °C. To determine how the mutation affected thermoregulation of the native pilus-adhesin transcription (that is, the *papI* and *papB-A* operons) we performed northern blot analyses of RNA extracted from *drdX*⁺ and *drdX*⁻ cells grown at 37 °C or 26 °C. As shown in Fig. 2, the level of *papI* mRNA in the *drdX* mutant strain was higher at both 37 °C and 26 °C than in the *drdX*⁺ strain at 37 °C. A similar, but less pronounced, derepressing effect by the *drdX* mutant was observed for the transcription of the *papB-A* operon. Recent studies suggested that the *papB* gene product is autoregulatory⁵ which explains why the *papB-A* transcription was not derepressed to the same extent as in the case of *papI*. The level of transcription from the *bla* gene of the plasmid vector was not altered by the *drdX* mutation at either high or low temperature (data not shown). Transcriptional analysis of the *drdX* gene itself (as defined below) showed that in wild-type bacteria there seemed that no difference was caused by the alteration of growth temperatures (Fig. 2c, lanes 1-4). The *drdX* gene transcript was about 550 nucleotides long, and our analysis showed that the gene was not transcribed at all in the *drdX* mutant strain.

To localize the mutant locus further we used a series of phage λ hybrid DNA clones from the *E. coli* gene library described by Kohara *et al.*⁶. The phage λ clones were tested in a marker rescue experiment. Two overlapping clones containing DNA sequences from the region around map position 27.5 min on the *E. coli* chromosome gave positive results in the test (Fig. 3). Phage clone 251 was used for further work and by subcloning different DNA fragments we obtained plasmids that could complement the *drdX* mutation *trans*. The plasmids pHMG400, pHMG401, and pHMG409 all restored normal growth at 30 °C, and it was evident that the *trans*-complementing activity resided within a 1-kb region (that is, the *StuI-EcoRI* fragment in pHMG409, Fig. 3). *Trans*-complementation by the *drdX*⁺ clones was also evident from the level of expression of the *pap-lacZ* operon fusion (Table 2).

The plasmids that complemented the *drdX* mutation all expressed a protein with an apparent relative molecular mass of about 16,000 (16K protein) (Fig. 3b and data not shown). Expression was lost upon replacing a *PstI-HpaI* fragment in plasmid pHMG409 with a fragment containing the *cat* gene (see

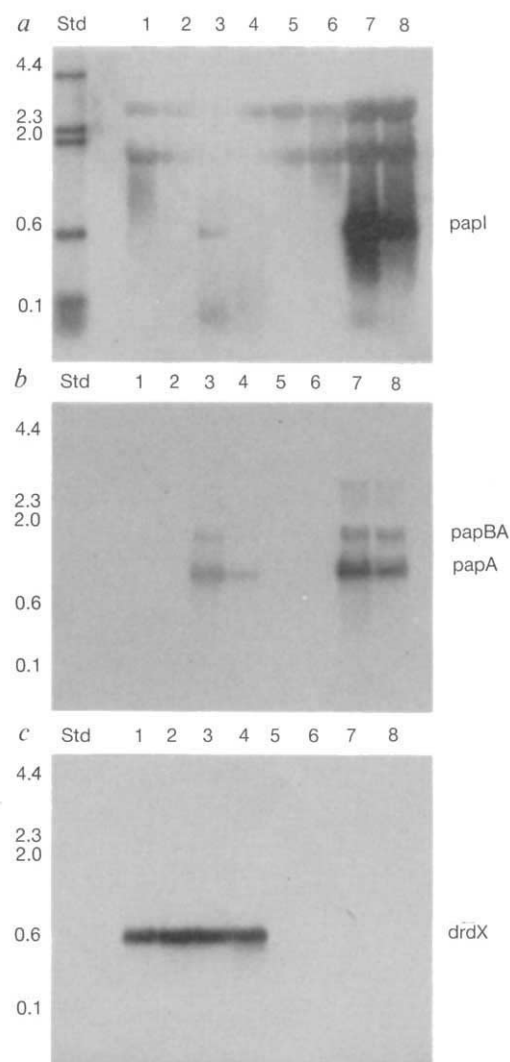


FIG. 2 Northern blot analysis of *pap* transcription by *drdX*⁺ and *drdX*⁻ *E. coli* at different growth temperatures. Lane 1: HMG11/pBR322 at 37 °C, Lane 2: HMG11/pBR322 at 26 °C, lane 3: HMG11/pPAP5 at 37 °C, lane 4: HMG11/pPAP5 at 26 °C, lane 5: HMG9/pBR322 at 37 °C, lane 6: HMG9/pBR322 at 26 °C, lane 7: HMG9/pPAP5 at 37 °C, lane 8: HMG9/pPAP5 at 26 °C. The *pap*⁺ plasmid pPAP5 carries the *EcoRI-BamHI* DNA fragment outlined in Fig. 1a (in the vector pBR322) and the probes for mRNA specified by the plasmid were described before^{1,3,21,27}. Equal amounts of total RNA (20 μ g) from the different cultures were used. The procedures for RNA extraction, blotting, and hybridization (using ³²P-labelled DNA fragments as probes) were as described recently^{1,21,27}.

TABLE 1 Levels of *papI* transcription in *drdX*⁺ and *drdX*⁻ strains of *E. coli**

Plasmid	Growth temperature	CAT specific activity in	
		HMG11(<i>drdX</i> ⁺)	HMG9(<i>drdX</i> ⁻)
pBEU310 (<i>papI-cat</i>)	37 °C	157 (135)	727 (694)
	26 °C	46 (3)	485 (408)
pKK232-8 (vector)	37 °C	22	33
	26 °C	43	77

* Strain HMG11 is a *recA*⁺ derivative of MC1029 (*drdX*⁺) and strain HMG9 is a *recA*⁺ derivative of HMG5 (*drdX*⁻). The specific activity of chloramphenicol acetyl transferase (CAT) was determined as described²⁶. The numbers within parentheses show the activity values after subtraction of the unit values obtained with the vector control.

TABLE 2 Effect of the *drdX* mutation and cloned *drdX* alleles on *pap-lacZ* expression and Bgl phenotype*

Plasmid	Host strain: MC1029 (<i>drdX</i> ⁺)		Host strain: HMG5 (<i>drdX</i> ⁻)	
	β -Gal (units) expression	Bgl phenotype	β -Gal (units) expression	Bgl phenotype
pLG339 (vector)	386	-	618	+
pHMG409 (<i>drdX</i> ⁺)	396	-	439	-
pHMG411 (<i>drdX:cat</i>)	396	-	643	+

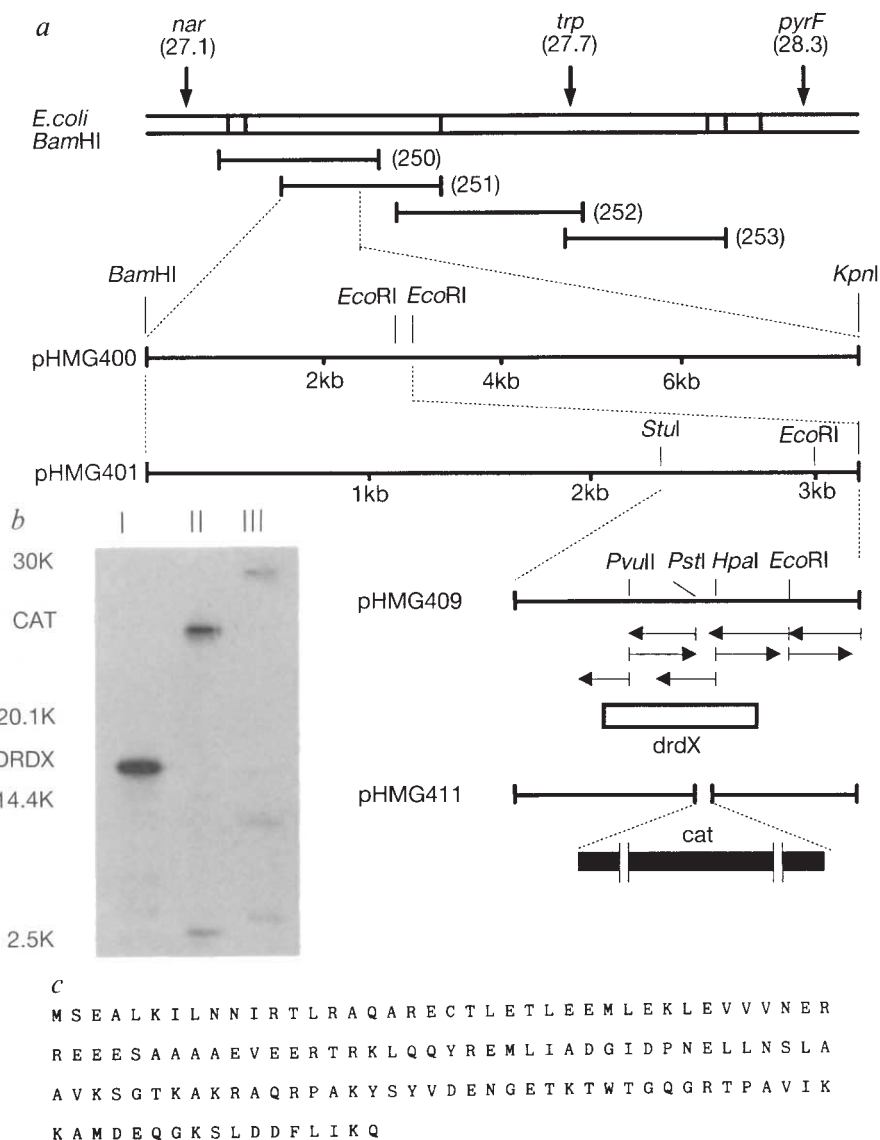
* The bacterial strains carried the *papI*⁺*papB*⁺*papA*:*lacZ* operon fusion plasmid pHMG1 (ref. 2) in addition to the plasmids indicated. Cells were grown at 37 °C. Assays for β -galactosidase specific activity were performed as described earlier (ref. 1). The Bgl phenotype was assayed on plates containing the chromogenic β -glucoside 5-bromo-4-chloro-3-indolyl β -D-glucopyranoside (Sigma).

plasmid pHMG411, Fig. 3a). DNA sequence analysis of plasmid pHMG409 revealed an open reading frame (ORF) for translation of a 15.5K protein. Translation of the ORF was abolished in plasmid pHMG411 and no other ORF was affected by the substitution. Determination of the mRNA size and start point (Fig. 2c, and unpublished data) showed that the *drdX*⁺ ORF is transcribed as a monocistronic operon. We conclude that the 15.5K ORF corresponds to the *drdX*⁺ gene product.

Comparison with published sequence data revealed that the deduced amino acid sequence of the *drdX* gene was completely identical to the protein sequence recently reported for a DNA-binding protein in *E. coli* called H-NS (Fig. 3c; ref. 7). A gene sequence (*hns*) corresponding to H-NS was also cloned and shown to originate from the region near map position 6.1 min on the *E. coli* chromosome⁸. The DNA sequence of the *drdX*⁺ ORF was identical to that of *hns* except for two positions but the differences do not cause amino-acid change. We have found both loci in many *E. coli* strains (unpublished data). Our results also demonstrate, however, that the *hns* locus is not transcribed in the *drdX* mutant strain (Fig. 2c). Some strains deleted for the *lac* operon are also lacking the *hns* region and we have found no indication that such deletions would alter *pap* expression^{1,2}. Consistent with the mRNA analysis (Fig. 2c), we found that the *drdX* mutation is caused by a deletion event abolishing transcription of the cistron (unpublished data). We conclude that the *drdX*⁺ locus is responsible for production of the protein in the *E. coli* strains we have studied.

The H-NS protein is generally accepted to be the same as the earlier described proteins called B1 (ref. 9), protein 16K (ref. 10), H1 (ref. 11), and H1a (ref. 12). The 20 amino acids determined at the amino-terminal end of protein 16K (ref. 10) exactly matched the sequence of the *drdX* gene product. The protein

FIG. 3 Genetic location and characterization of the *drdX* locus in *E. coli*. a, Diagrammatic segment of the *E. coli* chromosome with sites for the restriction endonuclease *Bam*HI (according to Kohara *et al.*⁶) indicated by vertical lines. The vertical arrows show position and coordinates of the *nar*, *trp*, and *pyrF* operons. The horizontal bars below show the extent of chromosomal DNA contained in λ vectors (clones 250–253) or plasmids (pHMG-clones). Cloning experiments were performed by standard recombinant DNA methodology²⁹ using the plasmid vectors pLG338 and pLG339³⁰. The horizontal arrows below pHMG409 indicate the region analysed by nucleotide sequencing and the box shows the position of the *drdX* open reading frame corresponding to a 15.5-kb protein. The thick, interrupted bar at the bottom symbolizes a 1.4 kb fragment carrying the *CAT* gene which was used for insertional inactivation in pHMG411. b, The inset shows an autoradiograph after SDS-PAGE analysis of [³⁵S]methionine-labelled extracts from *in vitro* transcription-translation experiments with pHMG409 (lane I), pHMG411 (lane II), and pLG339 (vector control, lane III) as DNA templates. The *in vitro* reactions were carried out with *E. coli* cell extracts (prokaryotic DNA-directed translation kit) according to the procedure suggested by the manufacturer (Amersham). c, The amino-acid sequence predicted from nucleotide sequencing of the *drdX* gene is shown in single-letter code.



shows properties associated with histone-like proteins and is found as a heat-stable protein in deoxyribonucleoprotein particles upon mild lysis of *E. coli* cells¹². There are about 20,000 protein molecules per cell and the dimer appears to be the predominant form. Purified protein binds tightly to double-stranded DNA under physiological conditions *in vitro* and increases the thermal stability of DNA. It may affect *in vitro* transcription and was shown to induce significant compaction of DNA¹². However, the role *in vivo* of this and other known histone-like bacterial proteins (for example, proteins HU and FirA) has largely remained elusive (see ref. 13 for a recent review). Recent genetic studies of protein HU and construction of mutants have confirmed that HU has a role in DNA replication and transposition^{14,15}.

The *drdX* mutation evidently affects expression of some other gene(s) in addition to the *pap* determinant. Mutations altering expression of some unlinked genes were earlier found to have a similar chromosomal map position in *E. coli*: *bglY* (ref. 16), *cur* (ref. 17), *pilG* (ref. 18), *osmZ* (ref. 19) and *virR* (ref. 20). We also found that the *drdX* mutant bacteria expressed a Bgl⁺ phenotype (which was complemented in *trans* to Bgl⁻ by the *DrdX*⁺ plasmid pHMG409; Table 2) as reported for the *bglY* and *osmZ* mutants^{16,19}. It is possible, although not yet proven, that the different mutations are allelic and that the *drdX* gene product influences the regulation of a set of *E. coli* operons.

The finding that a histone-like protein has a central role in temperature regulation of Pap pilus-adhesin expression in *E. coli* provided evidence for a novel mechanism of thermoregulation of transcription. The present results suggest that the protein negatively regulates two divergently oriented promoters. The intercistronic region between the *papI* and *papB* operons contains a divergent upstream activating sequence (UAS) which mediates the enhancing effect of cyclic AMP and of PapB on transcription of both promoters²¹. Our model implies that the interactions between the regulatory DNA region and the *drdX* gene product are different at high and low temperatures. A change in temperature may thereby cause altered conformational properties of DNA and could be the basis for differential protein-DNA interactions at different growth temperatures. Changes in the level of DNA supercoiling in the cell²², or more local alterations in topological microdomains²³, may influence such protein-DNA interactions and thereby modulate the effect on transcription from a particular promoter. Furthermore, a differential effect of protein H1 on the expression *in vitro* of ribosomal genes as a function of temperature was demonstrated earlier²⁴. The *in vitro* data on the histone-like function of the protein (ref. 12), mentioned above, and the multiple protein interactions (that is, involvement of PapI, PapB, catabolite repressor protein-cyclic AMP, in addition to RNA polymerase) in the present regulatory system, suggest that the DNA is engaged in a nucleoprotein structure resembling the enhancer complexes that regulate eukaryotic genes^{25,26}. The chromatin structures of eukaryotic and prokaryotic cells seem to be very different in that virtually all genes in bacteria appear available for transcription¹³. However, as shown here, we must also consider the possibility that there are genes in bacteria which are involved in nucleoprotein structures comparable to transcriptionally inactive chromatin and that transcriptional silencing is conditional in some instances. □

10. Laine, B., Sautiere, P., Spassky, A. & Rimsky, S. *Biochem. biophys. Res. Commun.* **119**, 1147–1153 (1984).
11. Cukier-Kahn, R., Jacquet, M. & Gros, F. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3643–3647 (1972).
12. Spassky, A., Rimsky, S., Garreau, H. & Buc, H. *Nucleic Acids Res.* **12**, 5321–5340 (1984).
13. Drica, K. & Rouvière-Yaniv, J. *Microbiol. Rev.* **51**, 301–319 (1987).
14. Wada, M., Kano, Y., Ogawa, T., Okazaki, T. & Imamoto, F. *J. molec. Biol.* **204**, 581–591 (1988).
15. Huisman, O. *et al. J. Bact.* **171**, 3704–3712 (1989).
16. Defez, R. & DeFelice, M. *Genetics* **97**, 11–25 (1981).
17. Diderichsen, B. *Molec. gen. Genet.* **180**, 425–428 (1980).
18. Spears, P. A., Schauer, D. & Orndorff, P. E. *J. Bact.* **168**, 179–185 (1986).
19. Higgins, C. F. *et al. Cell* **52**, 569–584 (1988).
20. Hromockyj, A. E. & Maurelli, A. T. *J. Bact.* **711**, 2879–2881 (1989).
21. Göransson, M., Forsman, K., Nilsson, P. & Uhlin, B. E. *Molec. Microbiol.* **3**, 1557–1565 (1989).
22. Pruss, G. J. & Drica, K. *Cell* **56**, 521–523 (1989).
23. Travers, A. *Nature* **341**, 184–185 (1989).
24. Travers, A. & Cukier-Kahn, R. *FEBS Lett.* **43**, 86–88 (1974).
25. Echols, H. *Science* **223**, 1050–1056 (1986).
26. Raibaud, O. *Molec. Microbiol.* **3**, 455–458 (1989).
27. Båga, M., Göransson, M., Normark, S. & Uhlin, B. E. *Cell* **52**, 197–206 (1988).
28. Lupski, J. R., Ruiz, A. A. & Godson, G. N. *Molec. gen. Genet.* **195**, 391–401 (1984).
29. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning. A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
30. Stoker, N. G., Fairweather, N. F. & Spratt, B. G. *Gene* **18**, 335–341 (1982).

ACKNOWLEDGEMENTS. We thank T. Edlund for suggestions during preparation of the manuscript. This work was supported by grants from the Swedish Natural Science Research Council and the National Swedish Board for Technical Development. M. G. was supported by a graduate fellowship grant from the Swedish Medical Research Council.

Role of a subdomain in the folding of bovine pancreatic trypsin inhibitor

Jonathan P. Staley*† & Peter S. Kim†‡

Departments of * Chemistry and ‡ Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

† Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, Massachusetts 02142, USA

THE disulphide-bonded intermediates that accumulate in the oxidative folding of bovine pancreatic trypsin inhibitor (BPTI) were characterized some time ago¹. Structural characterization of these intermediates would provide an explanation of the kinetically preferred pathways of folding for BPTI. When folding occurs under strongly oxidizing conditions, more than half the molecules become trapped in an intermediate, designated N*, which is similar to the native protein but lacks the 30–51 disulphide bond^{2–4}. We have tested the hypothesis⁵ that the precursor to N* is the one-disulphide intermediate [5–55], which contains the most stable disulphide in BPTI, and present evidence here that this is the case. A peptide model of [5–55], corresponding to a subdomain of BPTI, seems to fold into a native-like conformation, explaining why [5–55] does not lead to native protein and why it folds rapidly⁵ to N*. A native-like subdomain structure in a peptide model⁶ of [30–51], the other crucial one-disulphide intermediate, may explain the route by which [30–51] folds to native protein. Thus, much of the folding pathway⁵ of BPTI can be explained by the formation of a native-like subdomain in these two early intermediates. This suggests that a large part of the protein folding problem can be reduced to identifying and understanding subdomains of native proteins.

As part of an investigation of the BPTI folding pathway (Fig. 1a), we have designed a peptide model of [5–55] to determine whether it folds into a stable conformation in aqueous solution. Following a strategy used previously⁶ to design a peptide model of the intermediate [30–51], we designed our model, called PaPy, by assuming that the structure in [5–55] would be native-like and localized to a region near the 5–55 disulphide.

In native BPTI, the 5–55 disulphide joins the 3₁₀-helix to the α -helix, both of which contact the central β -sheet. PaPy includes residues from these three secondary structural units (Fig. 1b) and consists of two peptides, Pa and Py, joined by a disulphide bond corresponding to the 5–55 disulphide. Pa

Received 12 December 1989; accepted 19 February 1990.

1. Göransson, M., Forsman, K. & Uhlin, B. E. *Genes Dev.* **3**, 123–130 (1989).
2. Göransson, M. & Uhlin, B. E. *EMBO J.* **3**, 2885–2888 (1984).
3. Båga, M., Göransson, M., Normark, S. & Uhlin, B. E. *EMBO J.* **4**, 3887–3893 (1985).
4. Casadaban, M. J. & Cohen, S. N. *J. molec. Biol.* **138**, 179–207 (1980).
5. Forsman, K., Göransson, M. & Uhlin, B. E. *EMBO J.* **8**, 1271–1277 (1989).
6. Kohara, Y., Akiyama, K. & Isono, K. *Cell* **50**, 495–508 (1987).
7. Falconi, M., Gualtieri, M. T., La Teana, A., Losso, M. A. & Pon, C. L. *Molec. Microbiol.* **2**, 323–329 (1988).
8. Pon, C. L., Calogero, R. A. & Gualtieri, C. O. *Molec. gen. Genet.* **212**, 199–202 (1988).
9. Varshavsky, A. J., Nedospasov, S. A., Bakayev, V. V., Bakayeva, T. G. & Georgiev, G. P. *Nucleic Acids Res.* **4**, 2725–2745 (1977).

includes residues corresponding to the α -helix and a short β -strand. The residues in $P\gamma$ correspond to those in the 3_{10} -helix and the central β -sheet, which are joined by a linker of six glycines. The formation of the 30–51 disulphide was prevented by replacing Cys 30 and Cys 51 with alanines. To evaluate the importance of the central β -sheet region, we made a second

peptide model, called $P\alpha P\gamma(\Delta\beta)$, which lacks the β -sheet and glycine linker sequences (Fig. 1c).

$P\alpha P\gamma$ folds in aqueous solution, as shown by the sigmoidal transition observed for the temperature dependence of the circular dichroism (CD) signal at 222 nm (Fig. 2a). The transition indicates that $P\alpha P\gamma$ is essentially 100% folded at 0 °C and that

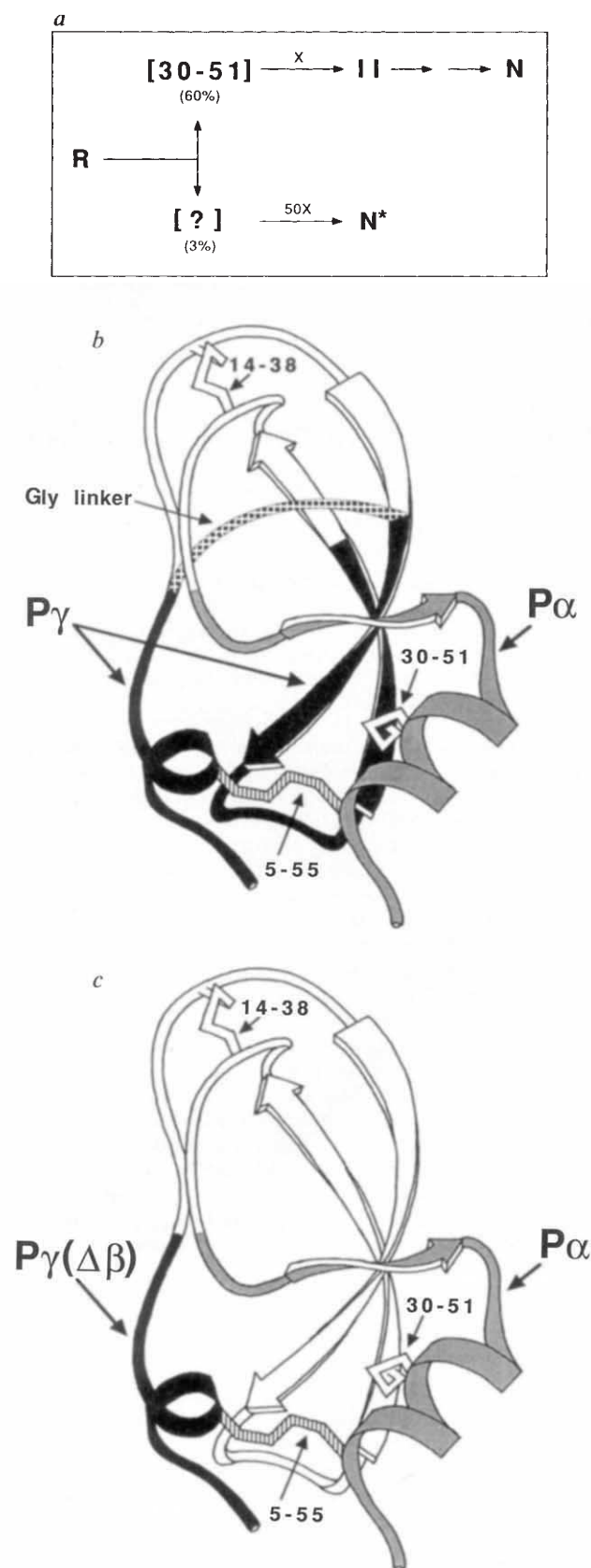


FIG. 1 Peptide models of the precursor to N^* . **a**, A simplified schematic of the folding pathway⁵ of BPTI at 25 °C, pH 8.7. Formation of both native BPTI and N^* results from a fork in the folding pathway at the one-disulphide stage of folding⁵. R refers to reduced BPTI; [30–51], to the one-disulphide intermediate containing a disulphide between Cys 30 and Cys 51; II, to the two-disulphide intermediates on the pathway to native BPTI; N, to native BPTI; N^* , to the native-like kinetically trapped intermediate; and [?], to the unidentified one-disulphide precursor to N^* . N^* is stable for days at 25 °C in the absence of reducing agents. Apart from reduction of the 30–51 disulphide, there are no major structural differences between N^* and native BPTI as judged by NMR³. Moreover, the X-ray crystal structure of a mutant of BPTI, with Cys 30 and Cys 51 replaced with alanines, reveals that the mutant has essentially a native fold⁴. The relative proportions of the one-disulphide intermediates are indicated⁵. As shown, [?] forms a second disulphide 50 times faster than [30–51]⁵, accounting for the significant accumulation of N^* from a poorly populated intermediate. **b**, Regions of BPTI included in $P\alpha P\gamma$ are indicated on a representation²⁰ of folded BPTI. The three native disulphides of BPTI are labelled. $P\alpha$, (shaded) includes residues 42–58: RNNFKSAEDAMRTCCGGA (one-letter amino acid code); Cys 51 has been substituted with Ala. (Note: $P\alpha$ of $P\alpha P\gamma$ differs slightly from $P\alpha$ of $P\alpha P\beta$ in that R42 is not included⁶ in $P\alpha P\beta$.) $P\gamma$ (black) includes residues 1–9, a linker of six glycines and residues 20–33: RPDFCLEPPGGGGGGRFYF-NAKAGLAQTF; Cys 30 has been substituted with Ala. The glycine linker (spotted) was used to join covalently residues 1–9 to 20–33. The disulphide between $P\alpha$ and $P\gamma$ corresponding to the 5–55 disulphide is striped. **c**, Regions of BPTI included in $P\alpha P\gamma(\Delta\beta)$ are indicated on a representation²⁰ of folded BPTI. $P\alpha$ is shaded. $P\gamma(\Delta\beta)$ (black) corresponds to residues 1–9: RPDFCLEPP, lacking the β -sheet and glycine linker sequences.

METHODS. Regions of BPTI important for stabilizing the 5–55 disulphide were defined by identifying residues involved in van der Waals' contacts and hydrogen bonds near the 5–55 disulphide in the structure^{21–23} of native BPTI. Peptides containing these residues were synthesized on an Applied Biosystems model 430A peptide synthesizer using 4[oxymethyl]phenylacetamidomethyl resins and standard reaction cycles, modified to include acetic anhydride capping. All peptides were cleaved from the resins using HF (Immunodynamics Inc., San Diego) except for $P\gamma(\Delta\beta)$ which was cleaved using trifluoromethane sulphonic acid. Peptides were desalted on a Sephadex G-10 column in 5% acetic acid and purified by high-pressure liquid chromatography on a Vydac C18 column using a linear water/acetonitrile gradient containing 0.1% trifluoroacetic acid. The identity of each peptide was confirmed by fast-atom bombardment mass spectrometry (at the NIH Mass Spectrometry Facility at MIT), yielding relative molecular masses of 1,826.4 for $P\alpha$ (calculated, 1,828.0), 3,046.3 for $P\gamma$ (calculated, 3,047.4) and 1,072.6 for $P\gamma(\Delta\beta)$ (calculated, 1,073.2). The heterodimeric disulphides were formed by activating one thiol with Ellman's reagent²⁴ dithio-bis-(2-nitrobenzoic) acid (DTNB). One volume of $P\alpha$ (1 mM in 1 mM HCl) was added dropwise to nine volumes of 25 mM DTNB, 0.1 M phosphate, 1 mM EDTA at pH 7.3, 23 °C. After 5 min, an equal volume of diethyl ether was added and the pH of the aqueous layer dropped to pH 2.0 by addition of 1 M HCl. The aqueous layer was washed seven times with ether to extract the DTNB. (Saturating freshly opened ether with water prevents oxidation of Met 52; T. G. Oas and J. S. Fetrow, unpublished results.) One equivalent of $P\gamma$ or $P\gamma(\Delta\beta)$ in an equal volume of 0.5% acetic acid, 1 mM EDTA was then added to the aqueous layer. The reaction was initiated by titrating to pH 7.3 with 0.75 M Na_2HPO_4 and quenched after 15 min by dropping the pH to pH 2.0 with 1 M HCl. The peptides were purified as described above. The identity of each was confirmed by mass spectrometry yielding relative molecular masses of 4,872.7 for $P\alpha P\gamma$ (calculated, 4,873.4) and 2,900.0 for $P\alpha P\gamma(\Delta\beta)$ (calculated, 2,899.2). Overall yield was ~45%. Glycine was used for the linker in $P\alpha P\gamma$ for maximum flexibility and minimum perturbation. The length of the linker was based on computer modelling. Linkers of different lengths were energy-minimized using the steepest descents algorithm of Discover (version 2.4, Biosym Technologies Inc., San Diego) in the presence of residues 1–9, 20–33 and 42–58 of BPTI. The coordinates of these residues were fixed in their native conformation²¹. The linker was required to make peptide bonds with residues 9 and 20. After minimization, linkers were evaluated by inspection of their backbone bond lengths using Insight (version 2.4, Biosym Technologies Inc.). Five glycines were sufficient to bridge residues 9 and 20 without perturbing native structure. Six glycines were used for additional flexibility.

the melting temperature is $\sim 28^\circ\text{C}$. A sigmoidal transition is not observed for reduced $\text{P}\alpha\text{P}\gamma$ or for oxidized $\text{P}\alpha\text{P}\gamma(\Delta\beta)$, indicating that both the disulphide (Fig. 2a) and the β -sheet sequence (Fig. 2b) are required for structure in $\text{P}\alpha\text{P}\gamma$.

The nuclear magnetic resonance (NMR) signals for protons in $\text{P}\alpha\text{P}\gamma$ are dispersed at low temperatures (Fig. 3a), providing

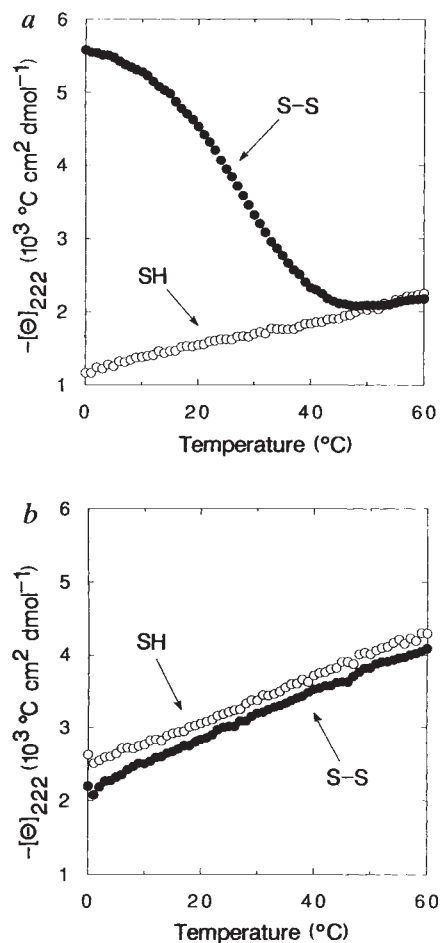


FIG. 2 $\text{P}\alpha\text{P}\gamma$ folds and requires the disulphide bond and the central β -sheet sequence for folding. *a*, Temperature dependence of $[\theta]_{222}$ for the oxidized (solid circles) and reduced (open circles) forms of $\text{P}\alpha\text{P}\gamma$. The melting temperature of oxidized $\text{P}\alpha\text{P}\gamma$ is $28 \pm 1^\circ\text{C}$, as determined by the maximum of the first derivative of the temperature dependence. Gel filtration studies indicate that $\text{P}\alpha\text{P}\gamma$ is a monomer under the conditions of these experiments. *b*, Temperature dependence of $[\theta]_{222}$ for the oxidized and reduced forms of $\text{P}\alpha\text{P}\gamma(\Delta\beta)$.

METHODS. Measurements were made with an Aviv Model 60DS circular dichroism spectrometer, equipped with an HP Model 89100A Peltier temperature control unit. A 10 mm pathlength cell was used, and all samples were degassed by vacuum. The concentration of peptide in all the experiments was $\sim 18 \mu\text{M}$. The concentrations of peptide stock solutions were determined by tyrosine and cystine absorbance²⁵ for $\text{P}\alpha\text{P}\gamma$ and by a ninhydrin assay²⁶ for $\text{P}\alpha\text{P}\gamma(\Delta\beta)$ (the ninhydrin colour yield of proline was determined as 7.3% at 570 nm). The sample buffer contained 40 mM sodium phosphate, 40 mM NaCl at pH 7.0. The reduced samples were generated in sample buffer, that also contained 0.2 mM dithiothreitol and 1 mM EDTA, by raising the pH temporarily to pH 8.7. Reduced samples were maintained under argon. Reverse-phase high-pressure liquid chromatography confirmed that each peptide was reduced before and after the circular dichroism experiments. The relative molecular mass (M_r) of $\text{P}\alpha\text{P}\gamma$ in sample buffer at 4°C was determined using a Sephadex G-25(f) column with the following standards: BPTI (M_r , 6,513), oxidized insulin B chain (M_r , 3,496), bombesin (M_r , 1,620), vitamin B_{12} (M_r , 1,355), human angiotensin I (M_r , 1,297) and oxytocin (M_r , 1,007). $\text{P}\alpha\text{P}\gamma$ was loaded at $20 \mu\text{M}$ and eluted at a peak concentration of $1 \mu\text{M}$. The apparent M_r of $\text{P}\alpha\text{P}\gamma$ was 4,900 (calculated 4,873) indicating that $\text{P}\alpha\text{P}\gamma$ is monomeric in these conditions. The apparent M_r was unchanged when $\text{P}\alpha\text{P}\gamma$ was loaded at 1 mM , eluting at a peak concentration of $50 \mu\text{M}$.

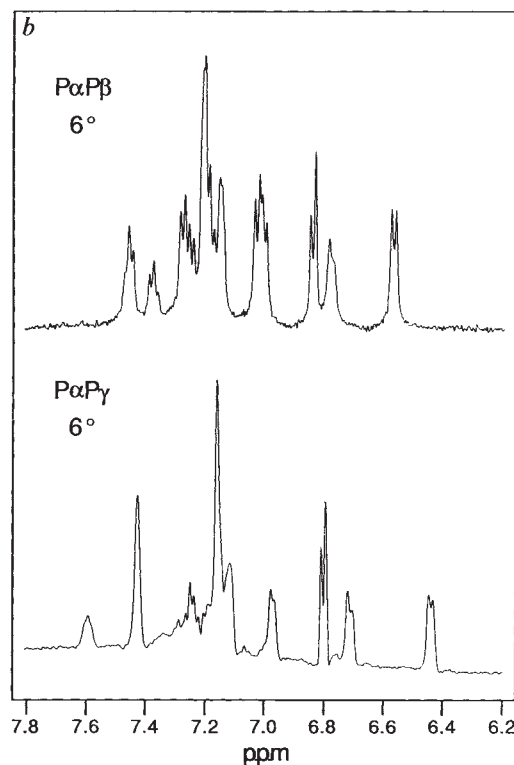
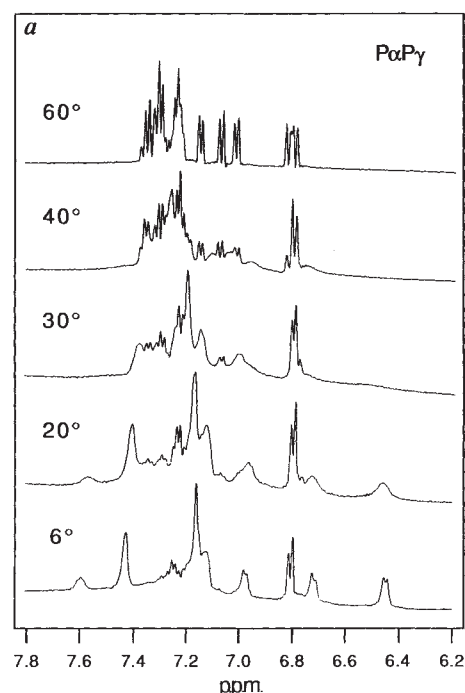


FIG. 3 Chemical shift dispersion in the aromatic region of the one-dimensional NMR spectrum of $\text{P}\alpha\text{P}\gamma$ depends on temperature and is similar to that of $\text{P}\alpha\text{P}\beta$. *a*, Temperature dependence of the aromatic region of $\text{P}\alpha\text{P}\gamma$. *b*, Comparison between the aromatic region of $\text{P}\alpha\text{P}\gamma$ and $\text{P}\alpha\text{P}\beta$ (ref. 6) at low temperatures.

METHODS. The spectra of $\text{P}\alpha\text{P}\gamma$ in 40 mM sodium phosphate, 40 mM NaCl at pH 7.0 in D_2O were collected, at a peptide concentration of 0.8 mM as determined by tyrosine and cystine absorbance²⁵. Trimethylsilylpropionic acid was used as a standard and its chemical shift at pH 7.0 was taken to be -0.018 p.p.m. (ref. 27). Data were collected on a 500 MHz spectrometer at the MIT Francis Bitter National Magnet Laboratory, presaturating residual HOD for 2 s. A shifted sine-bell apodization was used to enhance resolution.

further evidence that PaPy is folded. The pattern of dispersion is similar (Fig. 3b) to that observed for a peptide model of [30–51], called $\text{PaP}\beta$, which contains many of the residues in PaPy . As two-dimensional NMR showed that the structure of $\text{PaP}\beta$ was native-like (ref. 6; and T. G. Oas and P.S.K., unpublished results) and as a given pattern of dispersion is characteristic of a particular structure⁷, we came to the preliminary conclusion that the structure of PaPy is also native-like. Two-dimensional NMR studies of PaPy support this conclusion (unpublished results).

Four aromatic residues in native BPTI have reduced ring-flipping rates at low temperatures, as determined by NMR^{8,9}. Three of these residues are contained in PaPy . At least one of these aromatic rings, probably Phe 45, shows reduced flipping rates at low temperatures in PaPy (data not shown). Thus, the native-like structure in PaPy probably includes close-packed tertiary structure.

Given our demonstration that PaPy folds into a stable conformation, we concluded that [5–55] is the precursor to N^* (Fig. 4). Several observations support this conclusion. Firstly, [5–55], produced by folding BPTI in denaturing conditions, interconverts relatively slowly with the other one-disulphide intermediates under native conditions¹⁰, suggesting that [5–55] does fold. Secondly, [5–55] accumulates during the folding of circular BPTI, in which the two termini of BPTI are joined by a peptide bond¹¹. Thirdly, a mutant of BPTI, with Cys 30 and Cys 51 replaced by alanines, folds readily¹² to the equivalent of N^* , indicating that Cys 30 and Cys 51 are not required for the formation of N^* . Fourthly, peptide models of two other possible precursors to N^* , [14–38] (ref. 13) and [5–51] (unpublished results), are unfolded in aqueous solution at 0°C. Finally, of the three disulphides in native BPTI, removal of the 5–55 disulphide results in the greatest loss of stability⁵.

The native-like structure of PaPy provides a structural explanation for why [5–55] rapidly forms a second disulphide⁵ (Fig. 1a). The 14–38 disulphide lies at one end of the antiparallel β -sheet in native BPTI (Fig. 1b). Formation of the β -sheet would bring Cys 14 and Cys 38 close together and enhance disulphide formation. Moreover, linking the termini of the pro-

tein via the 5–55 disulphide and packing this region of the molecule against the β -sheet in a native-like way would constrain the loop regions containing Cys 14 and Cys 38 to be close together (Fig. 1b) and further enhance the rate of formation of the 14–38 disulphide.

Thus, whereas [30–51] is significant in the folding of BPTI because it is well populated^{10,14}, that is, thermodynamically stable, [5–55] is significant because in kinetic terms it favours the next step in folding⁵. A native-like structure does, however, appear to be responsible for both the thermodynamic stability of [30–51]⁶ and the rapid folding of [5–55] to N^* .

The native-like structure in PaPy also explains why [5–55] does not readily form the 30–51 disulphide to yield the intermediate [30–51, 5–55] (Fig. 4), as Cys 30 and Cys 51 would be buried. Similarly, these residues remain buried^{3,4} in N^* , explaining why N^* does not lead to native protein^{3,15}. Thus, the native-like structure in [5–55] seems to hinder the simple sequential formation of native disulphides in the branch of the BPTI folding pathway⁵ that leads to a dead-end intermediate. In the folding of proteins without disulphides, the burying of water molecules or ions may also hinder simple sequential acquisition of native structure; structural rearrangements may be required to release trapped water molecules or ions before folding to native protein can finish.

NMR studies of a peptide model for [30–51] indicate that the native-like structure in [30–51] leads to partial burying⁶ of Cys 55. This may explain why 5–55 does not form readily in [30–51] (Fig. 4) and why the branch of the BPTI folding pathway that leads to the native protein includes disulphide rearrangements through non-native intermediates (T. G. Oas and P.S.K., unpublished results; but see refs 16, 17).

It seems, therefore, that much of the apparently complex pathway for folding of BPTI can be explained by the formation of a native-like subdomain in the two early intermediates, [30–51] and [5–55]. We conclude that native-like subdomains are key determinants in the folding of BPTI. If this conclusion turns out to be a general one, then understanding the hierarchy of subdomains in native proteins^{18,19} will be an important part of understanding protein folding. □

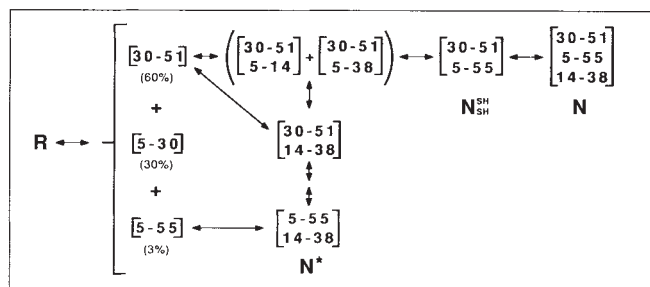


FIG. 4 Schematic diagram of the folding pathway⁵ of BPTI at 25°C, pH 8.7. R, N and N^* are as in Fig. 1. N^{SH} refers to the immediate precursor to native BPTI. Intermediates are designated by the disulphide bonds that they contain. The one-disulphide intermediates grouped together, are in rapid equilibrium on the minute timescale²⁸. It is interesting that [5–30], which contains a non-native disulphide, can serve as an intermediate in the rearrangement between [30–51] and [5–55]. The proportions of the one-disulphide intermediates are indicated⁵. The remaining 7% includes [30–55], [5–51] and other unidentified one-disulphide intermediates. The intermediates [30–51, 5–14] and [30–51, 5–38] are grouped together because both form from the one-disulphides, both form from [30–51, 14–38] and both rearrange to [30–51, 5–55] (ref. 16). Of the fifteen possible one-disulphide intermediates only [30–51], [5–30], [5–51] and [30–55] are observed in folding²⁸, but formation of N^* from these intermediates would require both disulphide bond formation and rearrangement, involving intermediates that have not been observed. N^* can form by rearrangement of a two-disulphide intermediate but this pathway accounts for only ~10% of N^* formed⁵. Molecules that fold to N^* are stable and kinetically trapped in strongly oxidizing conditions.

Received 10 November 1989; accepted 5 February 1990.

- Creighton, T. E. *Prog. Biophys. molec. Biol.* **33**, 231–297 (1978).
- States, D. J., Dobson, C. M., Karplus, M. & Creighton, T. E. *Nature* **286**, 630–632 (1980).
- States, D. J., Dobson, C. M., Karplus, M. & Creighton, T. E. *J. molec. Biol.* **174**, 411–418 (1984).
- Eigenbrot, C., Randal, M. & Kossiakoff, A. *Protein Engineering* (submitted).
- Creighton, T. E. & Goldenberg, D. P. *J. molec. Biol.* **179**, 497–526 (1984).
- Oas, T. G. & Kim, P. S. *Nature* **336**, 42–48 (1988).
- Pardi, A., Wagner, G. & Wüthrich, K. *Eur. J. Biochem.* **137**, 445–454 (1983).
- Wagner, G., DeMarco, A. & Wüthrich, K. *Biophys. struct. Mechanisms* **2**, 139–158 (1976).
- Wagner, G., Bruhwiler, D. & Wüthrich, K. *J. molec. Biol.* **196**, 227–231 (1987).
- Creighton, T. E. *J. molec. Biol.* **113**, 313–328 (1977).
- Goldenberg, D. P. & Creighton, T. E. *J. molec. Biol.* **179**, 527–545 (1984).
- Marks, C. B., Naderi, H., Kosen, P. A., Kuntz, I. D. & Anderson, S. in *Protein Structure, Folding and Design* Vol. 2 (ed. Oxender, D. L.) 335–340 (Liss, New York, 1987).
- Goodman, E. M. & Kim, P. S. in *Current Research in Protein Chemistry* (ed. Villafranca, J. J.) (Academic, New York, in the press).
- Creighton, T. E. *J. molec. Biol.* **87**, 579–602 (1974).
- States, D. J., Creighton, T. E., Dobson, C. M. & Karplus, M. *J. molec. Biol.* **195**, 731–739 (1987).
- Creighton, T. E. *J. molec. Biol.* **113**, 275–293 (1977).
- Creighton, T. E. *J. molec. Biol.* **151**, 211–213 (1981).
- Lesk, A. M. & Rose, G. D. *Biochemistry* **78**, 4304–4308 (1981).
- Richardson, J. S. *Adv. Protein Chem.* **34**, 167–339 (1981).
- Richardson, J. S. *Meth. Enzym.* **115**, 359–380 (1985).
- Deisenhofer, J. & Steigemann, W. *Acta crystallogr.* **B31**, 238–250 (1975).
- Wlodawer, A., Walter, J., Huber, R. & Sjölin, L. *J. molec. Biol.* **180**, 301–329 (1984).
- Wlodawer, A., Nacham, J., Gilliland, G. L., Gallagher, W. & Woodward, C. *J. molec. Biol.* **198**, 469–480 (1987).
- Ellman, G. *Arch. Biochem. Biophys.* **82**, 70–77 (1959).
- Edelhof, H. *Biochemistry* **6**, 1948–1954 (1967).
- Rosen, H. *Arch. Biochem. Biophys.* **67**, 10–15 (1975).
- DeMarco, A. *J. magn. Reson.* **26**, 527–528 (1977).
- Creighton, T. E. *J. molec. Biol.* **87**, 603–624 (1974).

ACKNOWLEDGEMENTS. We thank R. Rutkowski for assistance with synthesis and purification; J. Fetrov for help with computer graphics; T. Oas for help in identifying van der Waals' contacts and hydrogen bonds in BPTI; W. DeGrado for suggesting the use of DTNB in the synthesis of peptide pairs and R. Baldwin, T. Creighton, D. Goldenberg, E. Goodman, T. Oas and E. O'Shea for discussion. J. P. S. is a Howard Hughes Medical Institute predoctoral fellow. This research was supported by grants from the NIH and the Lucille P. Markey Charitable Trust.